University of Alberta

Supercritical Carbon Dioxide Fractionation of Lipids: Solubility Behavior to Process Development

by

Ozlem Guclu Ustundag ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial
fulfillment of the requirements for the degree of

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in

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Supercritical Carbon Dioxide Fractionation of Lipids: Solubility Behavior to Process Development** submitted by **Ozlem Guclu Ustundag** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Bioresource and Food Engineering**.

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For my mother and father,

Mine Güçlü and Musa Kazım Güçlü,

with gratitude and love…..

ABSTRACT

A systematic study on the solubility behavior of lipid components in $SCCO_2$ was carried out from binary to ternary and higher systems. The solubility trends of lipid components as affected by operating conditions and solute properties, the nature and extent of the deviation from binary behavior observed in multicomponent systems and the predictive ability of the binary data were determined. Also, the implications of the findings for fractionation processes were evaluated. Available literature binary solubility data of major (fatty acids, mono-, di- and triglycerides and fatty acid esters) and minor lipid components (stigmasterol, α-tocopherol, β-carotene and squalene) were correlated using Chrastil's equation and estimated model parameters were used to determine the solubility trends. The study of solubility behavior was hampered by the wide discrepancies between the experimental data and lack of phase behavior information, which is required for accurate interpretation of the solubility behavior such as melting behavior. The physical state of a solute was observed to have a significant impact on the solubility behavior; however, information on the melting behavior in $SCCO_2$ is far from complete and requires further research. Although binary solubility data provide valuable information on the relative solubility of lipid components and can be used to assess the potential of a fractionation process, its predictive ability is limited as the presence of other solutes affects the solubility behavior and thus the selectivity in multicomponent systems. General solubility trends can be established for ternary and higher systems by investigating the extent and nature of the deviation from binary behavior; however, further research is required to confirm the observed trends and determine the practical implications.

A fractionation column was designed and built to study the $SCCO_2$ column fractionation of fats and oils. Semi-continuous fractionation of canola oil deodorizer distillate was carried out to determine the feasibility of value-added processing of this feed material for the recovery of bioactive components such as sterols and tocopherols and to determine the effect of operating conditions (temperature, temperature gradient and pressure) on the yield and selectivity of the fractionation process. Sterol concentration as high as 40% (GC Area %) was achieved in the residue using a fractionation pressure of 25 MPa and a linear temperature gradient of 70-100 °C along the column. The findings indicate the potential of canola oil deodorizer distillate as a source of sterols and warrant further research on the countercurrent column fractionation to improve the separation efficiency.

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ABBREVIATIONS

DG	Diglyceride(s)
DHA	Docosahexaenoic acid
DO	Diolein
EPA	Eicosapentaenoic acid
FA	Fatty acid(s)
FAE	Fatty acid ester(s)
FAEE	Fatty acid ethyl ester(s)
FAME	Fatty acid methyl ester(s)
FFA	Free fatty acid(s)
GC	Gas chromatography
HBA	Hydroxybenzoic acid
LA	Linoleic acid
LSER	Linear Solvation Energy Relationship
LLL	Trilaurin
MeL	Methyl linoleate
MeM	Methyl myristate
MeO	Methyl oleate
MeP	Methyl palmitate
MG	Monoglyceride(s)
MMM	Trimyristin
MO	Monoolein
OA	Oleic acid
OOO	Triolein
P_c	Critical pressure
POO	Palmitoyl-dioleoylglycerol
PPO	Oleoyl-dipalmitoylglycerol
PPP	Tripalmitin
RRF	Relative response factor
$SCCO_2$	Supercritical carbon dioxide
SCF	Supercritical fluid
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
SSS	Tristearin
T_c	Critical temperature
TG	Triglyceride(s)
UCEP	Upper critical end point

NOMENCLATURE

Partition coefficient	Concentration of the solute in the vapor phase/ concentration in the liquid phase
Partition coefficient ratio	Ratio of partition coefficient in the multicomponent system to that in the binary system under the same operating conditions
Selectivity$_{A/B}$	Partition coefficient$_A$/partition coefficient$_B$
Cosolvent effect (solubility enhancement)	Ratio of solubility obtained with cosolvent addition to that without a cosolvent under the same operating conditions
Solubility ratio	Ratio of solubility in the multicomponent system to that in the binary system under the same operating conditions

1. INTRODUCTION AND LITERATURE REVIEW[1]

1.1. INTRODUCTION AND THESIS OBJECTIVES

Supercritical fluid (SCF) technology offers an attractive alternative for the processing of natural products and its commercial applications are growing worldwide. Successful application of the SCF technology to the processing of fats and oils requires reliable information on process fundamentals such as phase behavior and mass transfer as well as knowledge of the properties of solutes of interest. Totally predictive modeling of multicomponent phase behavior in SCFs has not been realized yet. Therefore, experimental solubility measurements play an essential role in both development of thermodynamic models and process design. Thus, the success of these modeling studies is in large part determined by the accuracy of the solubility data. Although solubility behavior of binary systems of lipids and supercritical CO_2 ($SCCO_2$) has been widely investigated, data on ternary and multicomponent systems are quite scarce. As well, interactions between lipid components of different classes and how they affect the solubility of each other when present in a multicomponent mixture are not well understood. No attempt has been made at a systematic in-depth analysis of the available data ranging from binary to ternary and multicomponent systems to establish solubility trends and determine implications for process development targeting fractionation of complex lipid mixtures that may be comprised of fatty acids (FA), fatty acid esters (FAE), mono-, di- and triglycerides (MG, DG, TG) and other minor lipid components.

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[1] A version of this chapter is to be published as a chapter in "Bailey's Industrial Oil and Fat Products, Sixth Edition" (Ed. F. Shahidi).

There is increasing recognition of the value of health benefiting compounds (nutraceuticals), such as tocopherols and sterols that are partially removed from vegetable oils during the refining process. Tocopherols are naturally occurring antioxidants in vegetable oils and a major source of vitamin E in the diet. Tocopherols and sterols find extensive application in food, cosmetic and pharmaceutical industries. Vegetable oil deodorizer distillate, which is a by-product of the conventional refining process, has been used as a source for the commercial production of sterols and tocopherols. It is a complex mixture containing aldehydes, ketones, hydrocarbons, free fatty acids (FFA), glycerides, sterols and tocopherols. Currently, deodorizer distillates of canola oil (which is the leading oil extracted and refined in Canada) produced in Canada are either sold to multinational companies for further processing or added back to the meal to be used as animal feed. Development of a process to concentrate the bioactive components of canola oil deodorizer distillate would benefit the Canadian oilseed processing industry, as it would enable value-added processing of this by-product in Canada. $SCCO_2$ fractionation of canola oil deodorizer distillate shows potential but it has not been studied. $SCCO_2$ fractionation of liquid materials is usually carried out using packed columns where fractionation is based on differences in the distribution of the mixture components between the extract and raffinate phases. This approach is anticipated to concentrate sterols in the raffinate phase but requires investigation. Therefore, the overall objectives of this thesis research were to carry out a systematic study to understand the solubility behavior of binary and ternary systems of lipid components and $SCCO_2$ and to determine the feasibility of $SCCO_2$ column fractionation for value-added processing of canola oil deodorizer distillate.

The specific objectives were:

a) to determine the general trends of binary solubility behavior of different lipid classes (FA, FAE, MG, DG, TG (Chapter 2), and minor lipids (β-carotene, sterols, tocopherols) (Chapter 3)) as affected by operating conditions and solute properties,

b) to study ternary systems of lipids in $SCCO_2$ (lipid + cosolvent + $SCCO_2$ (Chapter 4) and $lipid_1 + lipid_2 + SCCO_2$ (Chapter 5)) to determine general trends in ternary solubility behavior as affected by operating conditions and feed composition, to assess their implications for lipid fractionation, and to determine the nature and extent of deviations from binary behavior, and

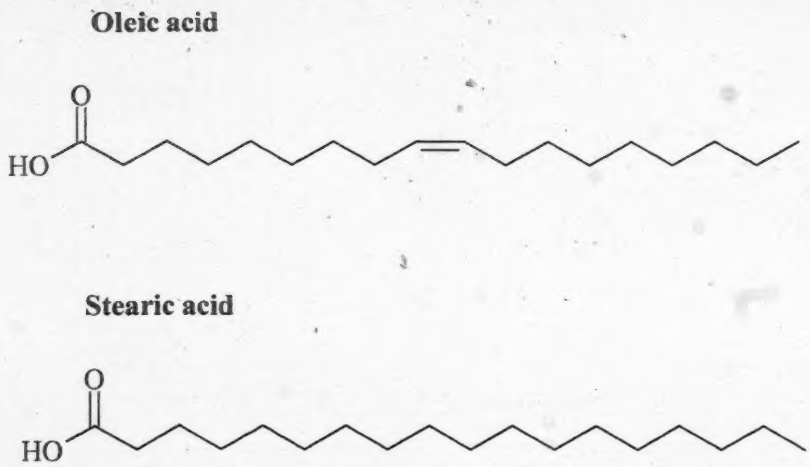
c) to design and build a $SCCO_2$ fractionation column and to study the effect of the operating variables on the efficiency of isolation of tocopherols and sterols from canola oil deodorizer distillate (Chapter 6).

1.2. LITERATURE REVIEW

1.2.1. Fats and oils

Fats and oils include a wide range of components, which are characterized by their solubility in organic solvents and insolubility in water. Fatty acids, fatty acid glycerides and fatty acid alkyl esters make up the major lipid classes, whereas pigments, sterols and vitamins such as tocopherols can be categorized as minor lipid components.

Fatty acids are aliphatic monocarboxylic acids found in lipids (Fig. 1.1). Their nomenclature is based on the number of carbon atoms and the number, location and geometric configuration (*cis* or *trans*) of the double bonds in their structure.


Oleic acid

Stearic acid

Figure 1.1. Structure of fatty acids (oleic and stearic acids).

Nomenclature of some common FA is given in Table 1.1. Oleic acid is the most common FA in nature, while short and medium chain acids (C4-C10) are present in milk fat and in a few seed fats, C12-24 acids are found in seed oils and animal fats and the long chain FA (up to C38) are common in waxes [1]. Fatty acids are esterified to glycerol to form glycerides. Mono-, di- and triglycerides are esters of glycerol and one, two and three FA, respectively (Fig. 1.2). Fatty acid ethyl and methyl esters (FAEE and FAME) are formed by the esterification of FA with ethanol and methanol, respectively (Fig. 1.3).

In a homologous FA series, the vapor pressure decreases and melting point increases with carbon number and hence molecular weight. Unsaturated acids and esters differ only slightly in vapor pressure from their saturated analogues. In a FA series with the same carbon number, melting points will be in the order: *cis*-olefinic acid<*trans* olefinic<saturated acid. The melting points of unsaturated FA depend on the nature, number, configuration and relative position of the double bonds. Alkyl esters of FA have lower melting points but show the same generalizations [1, 2]. A FA glyceride series

Table 1.1. Nomenclature of some common fatty acids.

Abbreviation[a]	Systematic name	Common name
12:0	Dodecanoic acid	Lauric
14:0	Tetradecanoic acid	Myristic
16:0	Hexadecanoic acid	Palmitic
18:0	Octadecanoic acid	Stearic
18:1 (ω-9)[b]	9-octadecenoic acid	Oleic
18:2 (ω-6)	9,12-octadecadienoic acid	Linoleic
18:3 (ω-3)	9,12,15-octadecatrienoic acid	Linolenic
20:5 (ω-3)	5, 8,11,14,17 eicosapentaenoic acid	EPA
22:6 (ω-3)	4,7,10,13,16,19-docosahexaenoic acid	DHA

[a] Carbon number: number of double bonds

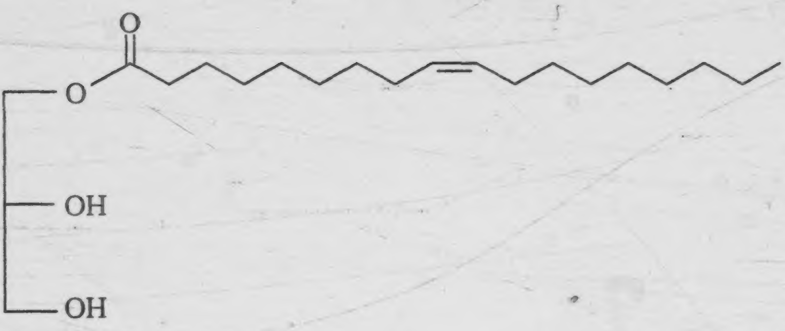
[b] Location of the first double bond from the methyl end of the molecule (ω carbon)

(MG, DG, TG) include components with varying molecular weights and polarities, which are reflected in their physical properties. Vapor pressures of components of a glyceride series follow the order: TG<DG<MG<FA [2, 3]. Physical properties of olein glyceride series are listed in Table 1.2. Fatty acids and their MG and DG can form H-bonds due to the presence of carboxyl and hydroxyl groups (Figs. 1.1 and 1.2), which can participate in H-bonding interactions both as an H-bond donor and acceptor.

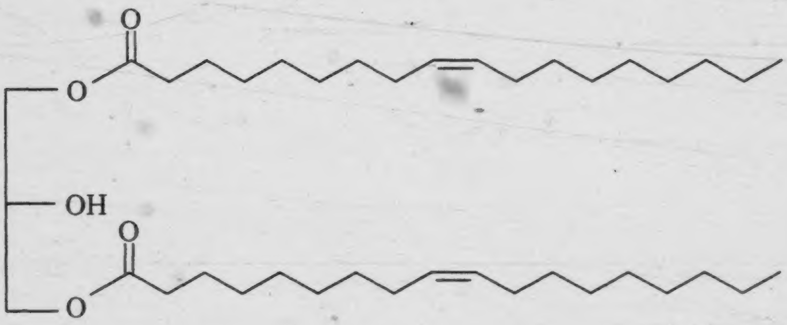
Separation methods based on differences in vapor pressure (such as distillation) can fractionate FA mixtures according to carbon number, whereas FA differing in the number of double bonds (such as stearic acid, oleic acid and linoleic acid) can not be separated by distillation. Due to their extremely low vapor pressures, TG can be distilled satisfactorily by molecular or short part distillation [2]. Solubility of FA in organic solvents decreases with increasing chain length and increases with unsaturation and is further affected by the position of double bonds and by their configuration (*trans* and conjugated less soluble than *cis* and non-conjugated) [1, 2].

Minor lipid components include a diverse group of components such as pigments (β-carotene), vitamins (tocopherols), sterols and hydrocarbons (squalene) (Table 1.3 and

Monoolein



Diolein



Triolein

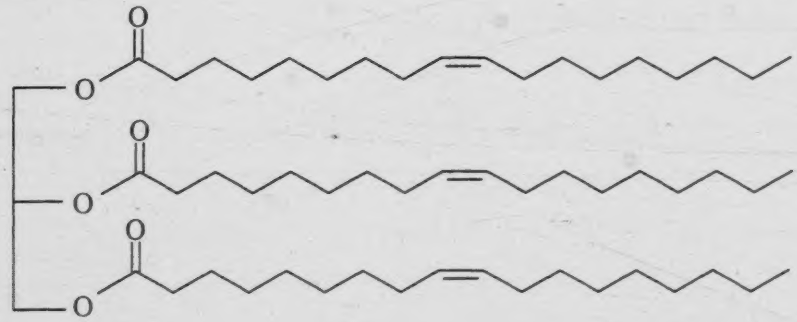
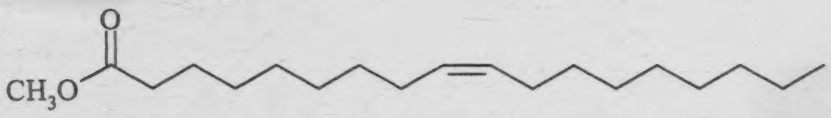


Figure 1.2. Structure of fatty acid glycerides (mono-, di-, and triolein).

Methyl oleate



Ethyl oleate

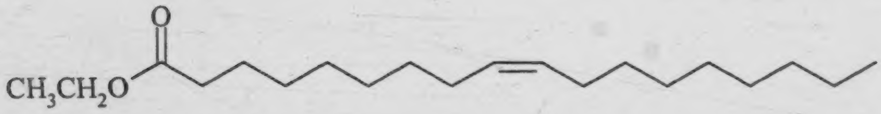


Figure 1.3. Structure of fatty acid alkyl esters (methyl oleate and ethyl oleate).

Figs. 1.4-1.7). Structure, properties and processing of these components will be covered in detail in Chapter 3.

Health benefits of minor lipid components such as tocopherols, sterols and certain FA (ω-3 FA (docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and α-linolenic acid), γ-linolenic acid and conjugated linoleic acid) have been widely investigated in recent years [5, 6]. Increasing evidence of health benefits of these lipid components coupled with changing consumer attitudes (increased awareness of diet-health link and increased tendency to self medicate), which is reflected in the considerable growth in the functional foods and nutraceutical market [7] have led to the reevaluation of conventional fats and oils processing for the recovery/concentration of these bioactive components. SCF technology has been increasingly used for the processing of nutraceuticals on a commercial level as it provides a solvent free, "natural" product which has a wide consumer appeal.

Table 1.2. Physical properties of major lipid components [2, 4].

Solute	Properties				
	Formula	MW	Melting point[a] (°C)	Boiling point (°C)	Solubility[b]
Fatty acids					
stearic acid	$C_{18}H_{36}O_2$	284.48	69.6	383[c]	very slightly sol in water, sol in alc, bz, chl, ace, carbon tetrachloride, carbon disulfide, amyl acetate, toluene
oleic acid	$C_{18}H_{34}O_2$	282.47	16.3	286[d]	prac insol in water, sol in alc, bz, chl, eth, fixed and volatile oils
linoleic acid	$C_{18}H_{32}O_2$	280.45	-6.5	230[e]	freely sol in eth, sol. in abs alc., miscible with dimethylformamide, fat solvents, oils
Monoglyceride					
monolein	$C_{21}H_{40}O_4$	356.6	35.2	186[f]	
Diglyceride					
diolein	$C_{39}H_{72}O_5$	621.0	21.5	-	
Triglyceride					
triolein	$C_{57}H_{104}O_6$	885.45	5.5	235-240[g]	practically insol in water, sol in chl, eth, carbon tetrachloride, slightly sol in alc
Fatty acid ester					
ethyl oleate	$C_{20}H_{38}O_2$	310.5		205-208[c]	miscible with alc, eth
methyl oleate	$C_{19}H_{36}O_2$	296.5	-19.8	168-170[h]	insol in water, miscible with anhydrous ethanol, eth

[a] Melting point of the highest melting, most stable polymorphic form
[b] Insol: insoluble; sol: soluble; bz: benzene; chl: chloroform; eth: ether; ace: acetone; alc: alcohol
[c] At 101.325 kPa
[d] At 13.332 kPa
[e] At 2.133 kPa
[f] At 0.027 kPa
[g] At 2.000 kPa
[h] At 0.267 kPa

Table 1.3. Physical properties of minor lipid components [4].

Solute	Properties				
	Formula	**MW**	**Melting point (°C)**	**Boiling point (°C)**	**Solubility**[c]
Pigment					
β-carotene	$C_{40}H_{56}$	536.85	183	-	sol. in CS_2, bz., chl.; moderately sol in eth., pet. eth., oils; very sparingly sol. in methanol, ethanol; practically insol. in H_2O, acids, alkalies.
Tocopherols					
α-tocopherol	$C_{29}H_{50}O_2$	430.69	2.5-3.5	200-220[a]	practically insol. in H_2O; freely sol. in oils, fats, ace., alc., chl., eth. and other fat solvents.
β-tocopherol	$C_{28}H_{48}O_2$	416.66	-	200-210[a]	insol. in H_2O; freely sol. in oils, fats, ace., alc., chl., eth. and other fat solvents.
δ-tocopherol	$C_{27}H_{46}O_2$	402.64	-	-	insol. in H_2O; freely sol. in oils, fats, ace., alc., chl., eth. and other fat solvents.
γ-tocopherol	$C_{28}H_{48}O_2$	416.66	-	200-210[a]	insol. in H_2O; freely sol. in oils, fats, ace., alc., chl., eth. and other fat solvents.
Sterols					
campesterol	$C_{28}H_{48}O$	400.66	157-158	-	
stigmasterol	$C_{29}H_{48}O$	412.67	170	-	insol. in H_2O, sol. in usual organic solvents.
β-sitosterol	$C_{29}H_{50}O$	414.69	140	-	
Hydrocarbon					
squalene	$C_{30}H_{50}$	410.70	-75	203[b]	practically insol. in H_2O; freely sol. in eth., petr. eth., CCl_4, ace., other fat solvents; sparingly sol. in alc., glacial acetic acid.

[a] At 0.0133 kPa

[b] At 0.0200 kPa

[c] Insol: insoluble; sol: soluble; bz: benzene; chl: chloroform; eth: ether; pet eth: petroleum ether; ace: acetone; alc: alcohol

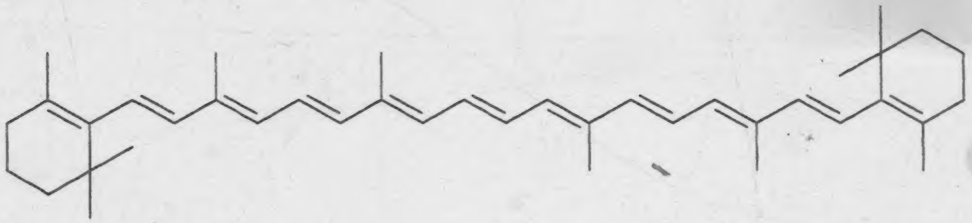
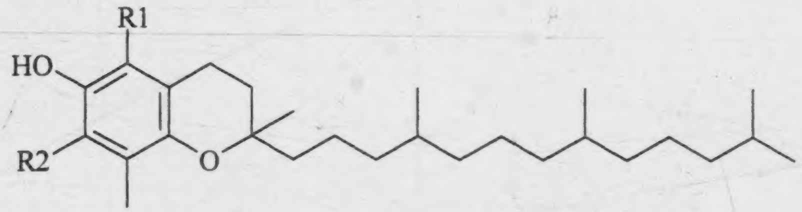


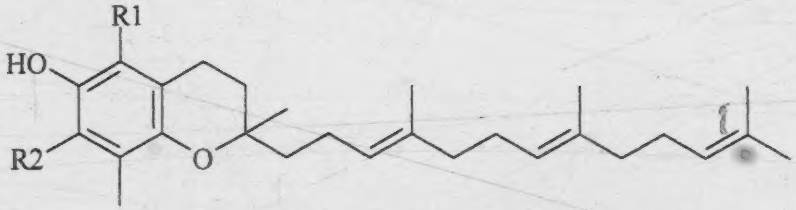
Figure 1.4. Structure of β-carotene.

Tocopherols



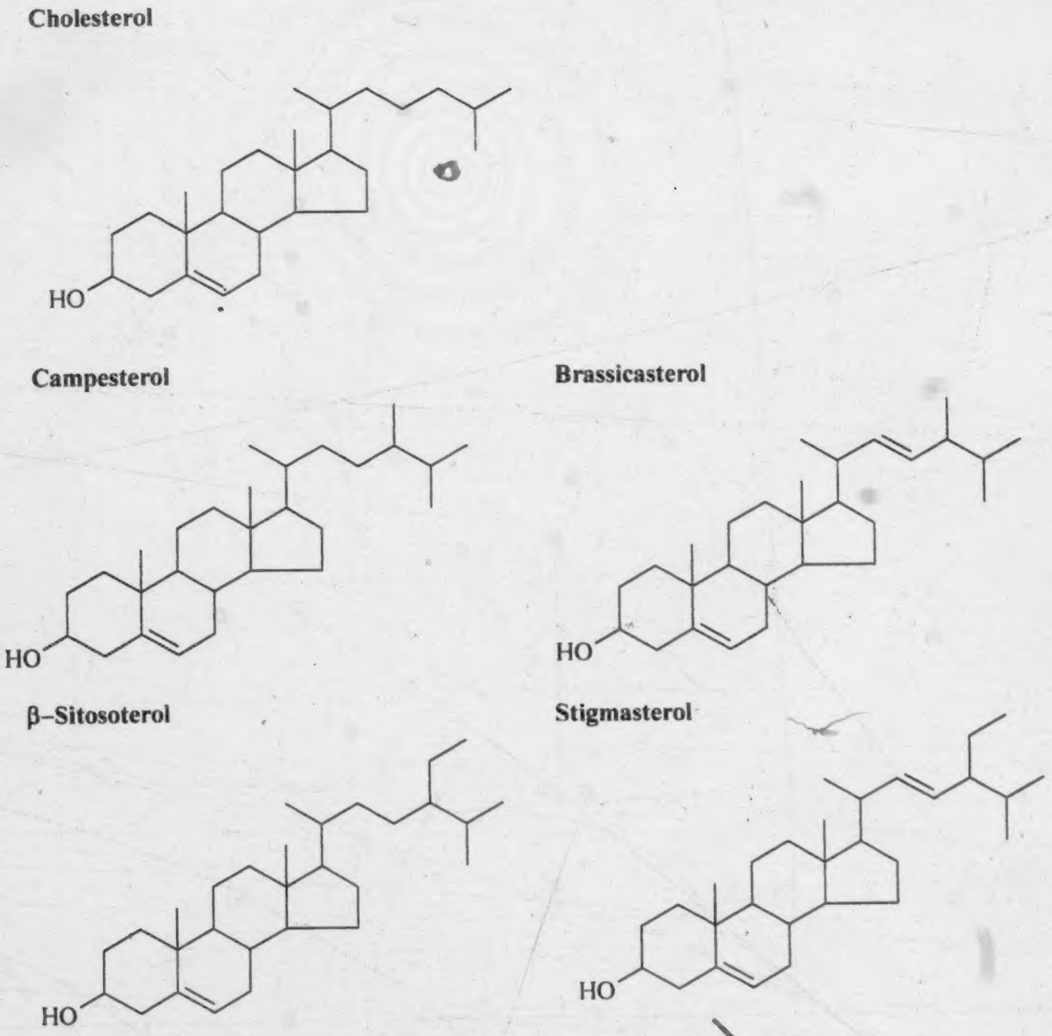
	R1	R2
α-tocopherol	CH3	CH3
β-tocopherol	CH3	H
δ-tocopherol	H	H
γ-tocopherol	H	CH3

Tocotrienols



	R1	R2
α-tocotrienol	CH3	CH3
β-tocotrienol	CH3	H
δ-tocotrienol	H	H
γ-tocotrienol	H	CH3

Figure 1.5. Structures of tocopherols and tocotrienols.


Cholesterol

Campesterol

Brassicasterol

β–Sitosoterol

Stigmasterol

Figure 1.6. Structures of phytosterols and cholesterol.

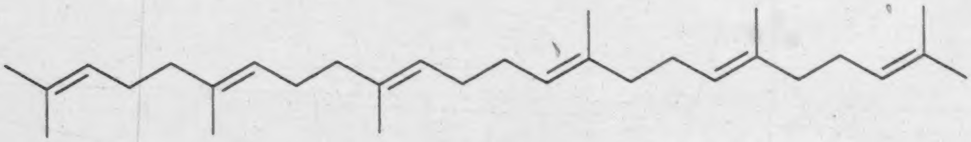


Figure 1.7. Structure of squalene.

1.2.2. Supercritical fluid technology

1.2.2.1. Definition and properties of supercritical fluids

A fluid is in its "supercritical state" at temperatures and pressures higher than its critical values (Fig. 1.8). Critical temperatures and pressures (T_c and P_c, respectively) of selected solvents are listed in Table 1.4. The critical point defines the highest pressure and temperature, at which gas and liquid phases can coexist. As the critical point is approached the distinction between the gaseous and liquid phases diminishes such that their properties are identical at the critical point.

SCFs are attractive solvents as they exhibit physicochemical properties intermediate between those of liquids and gases (Table 1.5). The density, thus the solvating power of a SCF approaches that of a liquid, whereas the diffusivity and viscosity are intermediate between gas-like and liquid-like values, resulting in faster mass transport capacity [9]. Due to the large compressibility near their critical points, their densities/solvent power can be varied by changes through operating conditions (temperature and pressure), resulting in operational flexibility, which can be exploited to achieve the required separation.

CO_2 is the solvent of choice in food applications as it is an inert, non-toxic, non-flammable, environmentally friendly solvent with a moderate critical temperature (31 °C) and pressure (7.4 MPa), which is readily available in high purity and low cost [10].

1.2.2.2. History and commercial applications

The discovery of the critical point of substances dates back to 1820s when Cagniard de la Tour [11] observed the disappearance of the gas-liquid meniscus at temperatures higher than critical values under pressure. The solvent power of SCFs has

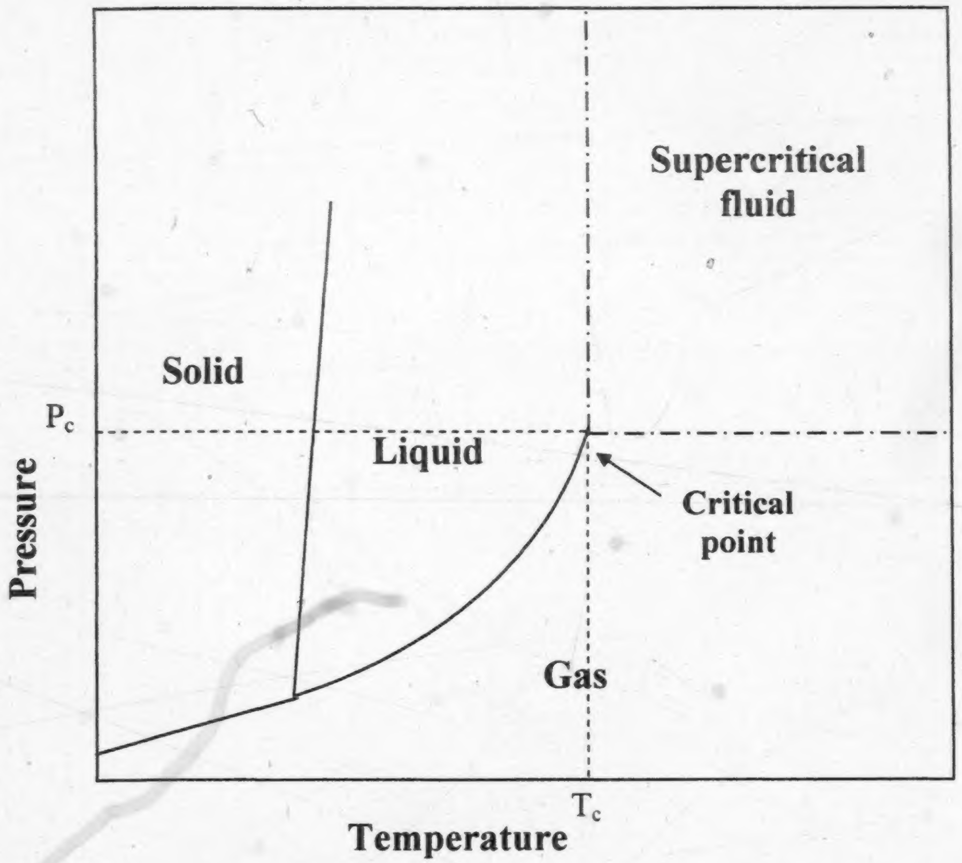

Supercritical fluid
Solid
P_c
Liquid
Critical point
Pressure
Gas
T_c
Temperature

Figure 1.8. A typical phase diagram.

been reported as early as 1879 [12]. Although potential applications of nearcritical fluids (SCFs and liquefied gases) had been proposed since the 1930s for various extraction and separation processes such as the separation of high molecular weight mixtures [13], de-asphalting of petroleum [14] and purification of fatty oils [15], it was the work of Zosel [16] at the Max-Planck Institute in Mannheim, Germany that brought this technology into commercial focus in 1960's, which eventually led to its commercialization for coffee decaffeination and hops extraction purposes in Germany in the late 70's and early 80's.

Table 1.4. Critical points of selected solvents [8].

Solvent	**T_c (K)**	**P_c (MPa)**
Carbon Dioxide	304	7.38
Ethane	305	4.88
Ethylene	282	5.03
Propane	370	4.24
Propylene	365	4.62
Methanol	513	8.09
Acetone	508	4.70
Benzene	562	4.89
Toluene	592	4.11
Ammonia	406	11.3
Water	647	22.0

Table 1.5. Properties of gases, liquids and supercritical fluids [9].

	Density (g/mL)	**Viscosity (g/cm s)**	**Diffusivity[a] (cm^2/s)**
Gas 0.101325 MPa, ~15-30 °C	$(0.6-2)*10^{-3}$	$(1-3)*10^{-4}$	0.1-0.4
Supercritical fluid T_c, P_c	0.2-0.5	$(1-3)*10^{-4}$	$0.7*10^{-3}$
~T_c, 4P_c	0.4-0.9	$(3-9)*10^{-4}$	$0.2*10^{-3}$
Liquid ~15-30 C	0.6-1.6	$(0.2-3)*10^{-2}$	$(0.2-2)*10^{-5}$

[a] Self diffusion for gas and SCF, binary mixture for liquid

Since then a number of supercritical fluid extraction (SFE) plants have been built around the world in varying sizes (ranging from 4 L to 6500 L) for the processing of natural products such as hops, tobacco, spices and herbs, aromas and nutraceuticals [17-21]. In addition to extraction processes, applications of SCF technology has widened in recent years to include particle design, coating, aerosols, impregnation, cleaning, supercritical water oxidation, analytical extraction and chromatography, production scale chromatography, extrusion, nucleation, infiltration of materials into polymers and chemical reactions [19, 21].

Motivations for the commercialization of SCF technology included concerns over the use of organic solvents, which was reflected in tightening government regulations (for example, prohibition of the use of methylene chloride for coffee decaffeination in Germany was the driving force behind the commercialization of coffee decaffeination technology), changing consumer attitudes, improved product quality, increased demands on product performance and development of innovative products or processes [18, 20]. SFE has been increasingly used in recent years around the world for the processing of nutraceuticals as a "natural" alternative to traditional extraction processes. The ability to claim "natural extracts" in marketing these products is a significant advantage in today's marketplace. SFE also offers the advantage of mild operating conditions for heat sensitive compounds (compared to distillation), and a solvent-free extract and residue (compared to solvent extraction). It provides an oxygen-free environment and thus limits oxidative degradation of the product.

1.2.2.3. Supercritical fluid processing of fats and oils

1.2.2.3.1. Fundamentals of supercritical fluid extraction

Successful application of SCF technology to any separation process requires information on the solubility behavior of the solutes of interest as affected by operating conditions and solute properties. Binary solubility of lipid components in $SCCO_2$ has been investigated by various researchers [22-24]. Multicomponent data, though relatively scarce, are available for a number of systems such as FAE mixtures, deodorizer distillates and vegetable oils [25-29]. No attempt has been made at a systematic analysis to establish the general solubility trends of minor and major lipid components. Solubility behavior of minor and major lipid components in $SCCO_2$ will be covered in great detail in the following chapters (Chapters 2-5).

As in any extraction process, SFE kinetics is made up of three periods; solubility controlled or constant extraction rate period, transition period and diffusion controlled or falling rate period (Fig. 1.9). During the initial period, the solute readily available on sample surface is extracted/solubilized in the solvent and the extraction rate is constant. The slope of the extraction curve, weight of extract versus volume or weight of CO_2 gives the loading of the solvent. This value corresponds to solubility only if equilibrium is attained during extraction by operating at low enough solvent flow rates. The extraction rate starts to decline in the transition period after which the extraction is controlled by diffusion of the solute and solvent in the sample matrix. Efficiency of SFE of any feed material is thus affected by operating conditions and feed material properties; therefore, both should be optimized for an efficient process. Flaking has been shown to be the most efficient pretreatment for the SFE of oilseeds [30-33]. Mass transfer modeling of SFE of oilseeds has been studied by various researchers [33-35].

The SFE process consists of two basic steps: extraction and separation. During the extraction step, the soluble material is extracted under high pressure from the solid substance matrix and transported away by the solvent. Separation of the supercritical solvent from the extract (regeneration of the supercritical solvent and recovery of the solute) can be achieved by reducing the density/solvent power of the SCF solvent by decreasing the pressure or elevating the temperature or both. The process must be adapted to the separation problem at hand, considering the target material [36].

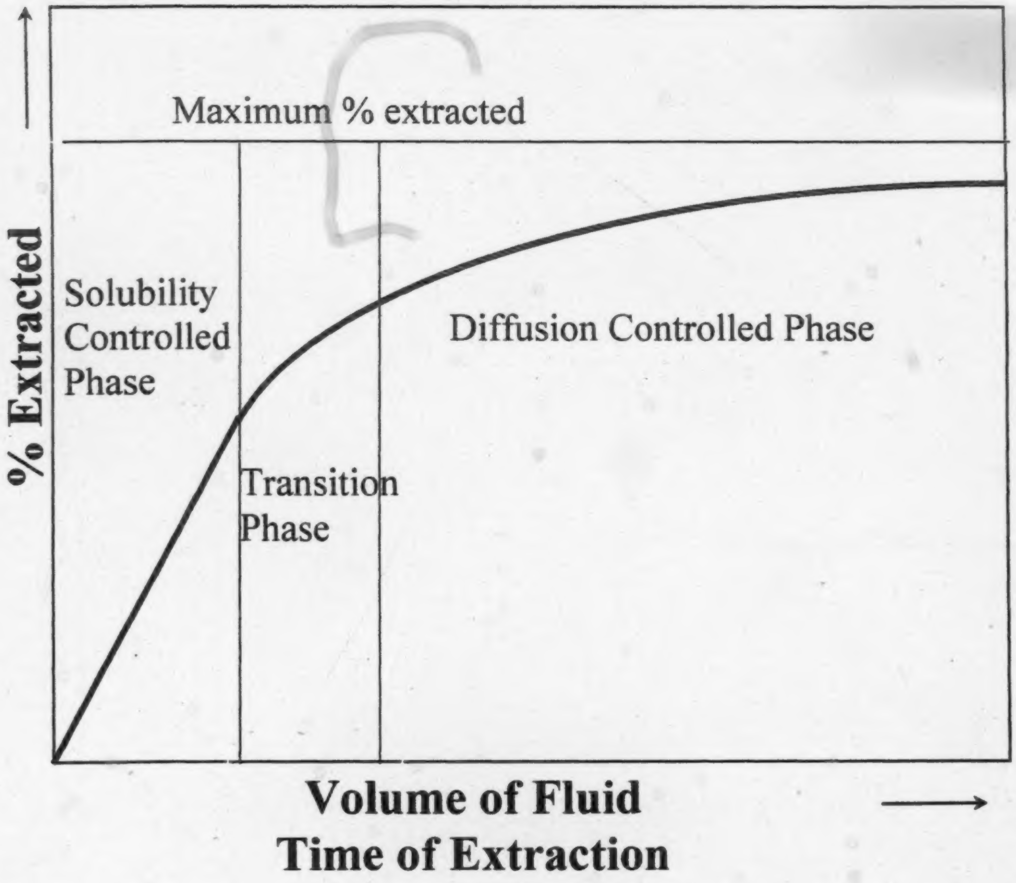

Maximum % extracted
Solubility Controlled Phase
Transition Phase
Diffusion Controlled Phase
% Extracted
Volume of Fluid
Time of Extraction

Figure 1.9. A typical supercritical fluid extraction curve.

The process can be operated on batch, semi-continuous or continuous mode [37]. Batch processing (Fig. 1.10) involves contacting a batch of solid feed material with a continuous solvent stream. However, the necessity to depressurize the vessel for the introduction of a new batch of material limits the efficiency of the process. Therefore, most production plants are operated semi-continuously where the extraction volume is spread over three or four vessels, which are operated in a cycle such that while one vessel is being extracted another is being loaded and a third vessel is being

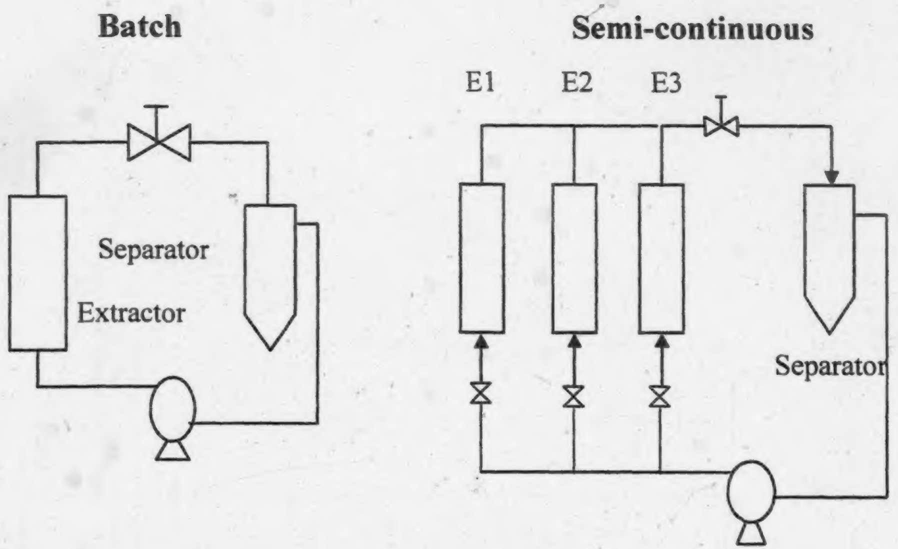


Figure 1.10. Typical supercritical fluid extraction systems.

pressurized/depressurized (Fig. 1.10). Continuous introduction of the solid feed material into the extraction vessel under high pressure poses a processing challenge. Lock hopper vessels and screw conveyors are some alternatives being investigated for the continuous processing of solid feed material under high pressure [38].

1.2.2.3.2. Fundamentals of supercritical fluid fractionation

In SCF fractionation, separation is based on differences in solubility behavior of mixture components arising from both the differences in component volatilities (as in distillation) and the differences in intermolecular interactions between the mixture components and the SCF solvent (as in solvent extraction) [16].

SCF fractionation of extracts can be achieved by fractional extraction or fractional separation. During SFE, fractionation occurs with time, which is reflected in the compositional differences of the fractions collected throughout the process as a function of time. Fractional extraction, thus relies on compositional differences in fractions throughout extraction. It is governed by the differences in the solubility behavior of extract components but may also be affected by the location of the solutes in the solid sample matrix. During extraction of vegetable oils, for example, fractionation of minor components such as phospholipids, sterols and tocopherols occurs as evidenced by higher concentrations in the later fractions [39]. Concentration of FA has also been observed during $SCCO_2$ extraction of oilseeds [39, 40]. Fatty acids are usually concentrated in the earlier fractions due to their higher solubility compared to that of TG [40]; however, Friedrich et al. [39] observed increasing concentrations of FA in the later fractions during soybean oil extraction. Eggers [32] observed that a higher amount of water was extracted than oil towards the end of $SCCO_2$ extraction of rapeseed press cake and attributed it to the higher transport resistance of oil than water in the cake. The compositional differences between fractions can be accentuated by using a density gradient, which can be achieved by increasing the pressure or adding a cosolvent at certain time intervals during extraction. In fractional separation (Fig. 1.11), which exploits the differences in the solubility behavior of extract components, fractionation is achieved after the extraction step using a series of separators operated at conditions adjusted to yield a stepwise decrease in solvent power/density.

Supercritical fractionation of a liquid feed material is usually carried out in a packed column (Fig. 1.11). Such columns are not available commercially and have to be custom built either in-house or by manufacturers of extraction units. Lab-scale and pilot-scale supercritical columns, 0.61-13.6 m high with internal diameters of 14.3-68 mm are available in research labs around the world and have been used for the processing of

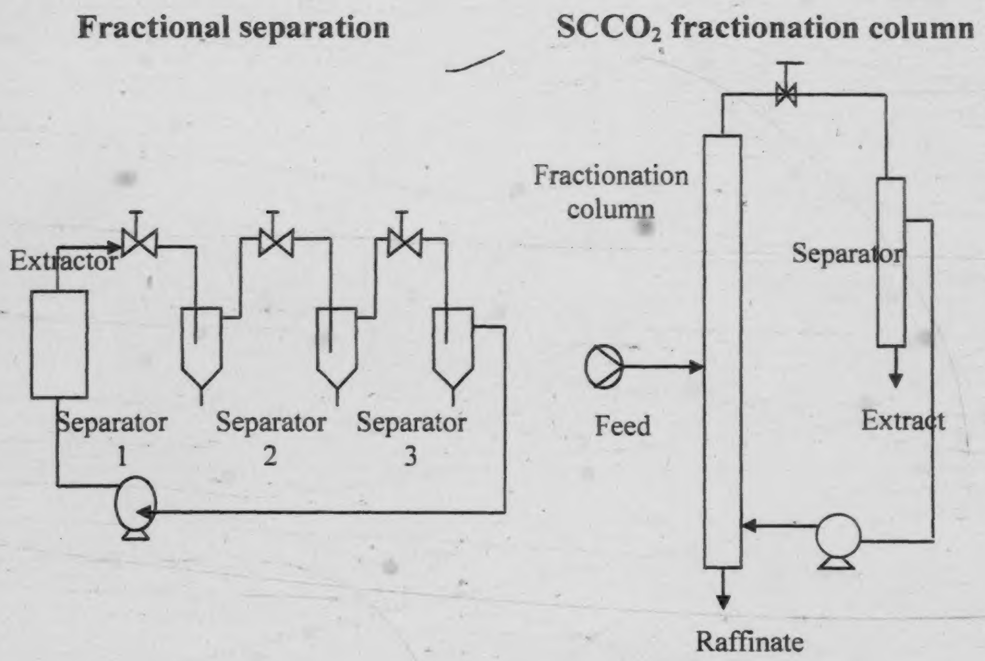

Fractional separation
SCCO$_2$ fractionation column
Extractor
Separator 1
Separator 2
Separator 3
Fractionation column
Separator
Feed
Extract
Raffinate

Figure 1.11. Typical supercritical fluid fractionation modes.

deodorizer distillates [41-47], vegetable and fish oils [48-62], cocoa butter and milk fat [63-66]. Packed columns can be operated in a continuous mode by introducing the liquid feed material into the column using a feed pump to achieve a co-current or countercurrent operation. A thermal gradient along the column can be applied to generate an internal reflux such that feed components are subjected to higher temperatures/lower solvent densities as they move up the column resulting in less soluble components to drop out. While in earlier designs, the thermal gradient was achieved using a hot finger mounted at the top of the column [16, 67], in more recent designs, the thermal gradient is achieved by independent temperature control of column zones [53]. An external reflux can be generated by adding an external reflux pump [68].

1.2.2.3.3. Applications in processing of fats and oils

The ability to fine tune solvent properties of SCFs through changes in operating conditions can be exploited in a wide range of applications such as separation and fractionation processes, where flexibility in process implementation offers the researcher/processor a gamut of possibilities.

Earlier work on supercritical fluid processing of natural fats and oils as reviewed by Brunner and Peter [69] included extraction of oil from oilseeds, extraction of fat from egg yolk and starch containing vegetable matter, extraction of lanoline from wool grease, refining of natural oils, purification of MG, and fractionation of cod liver oil using a variety of solvents such as propane, propylene, ethylene, CO_2 and ethane.

SCF processing of fats and oils have been widely investigated in the last three decades. While the earlier work focused on the extraction of commodity oils such as soybean and canola [33, 39], extraction of specialty oils (oils high in bioactive components), and fractionation of fats and oil mixtures [44, 46] have been the subject of more recent studies.

1.2.2.3.3.1. $SCCO_2$ extraction of vegetable oils

$SCCO_2$ extraction of oilseeds has been the focus of increased research activity in the 1980s when a variety of oilseeds such as soybean, cottonseed, corn germ, rapeseed and sunflower has been extracted using $SCCO_2$ [70]. While $SCCO_2$ extracted oils were shown to have similar quality compared to hexane extracts, they had lighter color and lower iron and phospholipid contents resulting in a lower refining loss and simplification of the subsequent refining steps [71-74]. However, the oxidative stability of CO_2 extracts was lower than hexane-extracted oils, which was attributed to the low phospholipid content of the CO_2 extracts [75, 76]. Protein quality of the CO_2-extracted meal was comparable to that of hexane-extracted meal [77]. In spite of the high volume of research carried out on extraction of oilseeds, commercial applications of $SCCO_2$ extraction of oilseeds has not been realized yet. High equipment costs associated with the process and the inability to achieve continuous processing of high volumes of oilseeds have been cited as the major impediments to commercialization to date [38]. However, recent developments in equipment design may make this a reality in the near future.

In the case of specialty oils, the cost of SFE can be balanced by the high value of the product and the added advantage of natural processing. Rice bran [78-81] and corn fiber oils [82] are receiving a lot of attention due to their high sterol content. Cereals such as wheat germ [83, 84] and barley [85] in addition to plants such as *Silybum marianum* (milk thistle) [86] have also been investigated as sources for the production of oils rich in tocopherols. $SCCO_2$ extraction of oils rich in carotenes from sources such as palm fruit and palm oil fibers [87, 88] have also been reported. In the case of highly unsaturated oils such as evening primrose oil [89-91] and flaxseed oil [92], which are good sources of α- and γ-linolenic acids, respectively, the ability to operate at mild operating conditions and in an oxygen-free environment is a significant advantage of $SCCO_2$ processing.

1.2.2.3.3.2. $SCCO_2$ fractionation of fats and oils

$SCCO_2$ fractionation of fats and oils have been investigated by various researchers for the refining (deacidification and degumming) of oils, for the concentration of bioactive components of fats and oils and by-products and for the fractionation of milk fat.

$SCCO_2$ processing has been investigated as an alternative to traditional oil refining processes for various oils, such as palm oil [50, 93], rice bran oil [49, 53, 79], olive oil [48, 60], black cumin seed oil [94], peanut oil [51] and soybean oil [95]. Dunford and King [49] achieved deacidification of rice bran oil to a level of <1% FFA while retaining free sterol and oryzanol contents at 0.35% and 1.8%, respectively, using a $SCCO_2$ fractionation column. Ooi et al. [50] studied refining of palm oil using continuous column fractionation and fractional separation with three separators and reported that the addition of a cosolvent (ethanol) improved the refining process. List et al. [95] utilized the low solubility of phospholipids in $SCCO_2$ to achieve degumming of soybean oil.

By-products of conventional oil production and refining have been investigated as raw materials for the concentration of bioactive components. Birtigh et al. [96] investigated SFE of carotenes and tocopherols from waste products of palm oil production, residue of mechanical processing and palm leaves. Ibanez et al. [97] studied the separation of tocopherols from olive by-products using fractional separation with two separators. While fractions from the first separator contained TG, waxes and sterols, tocopherols were concentrated in the second separator.

SFE, fractional extraction, fractional separation and column fractionation have been used to concentrate the bioactive components of deodorizer distillates. During SFE of deodorizer distillates [46, 98, 99], FFA were preferentially extracted while tocopherols and sterols were enriched in the raffinate. Lee et al. [98] concentrated the tocopherols in the sterol-free, esterified soybean distillate (containing 18 wt% tocopherols) up to 40 wt% using $SCCO_2$ extraction and concluded that a countercurrent multistage column was required to achieve a higher tocopherol concentration. Chang et al. [100] studied $SCCO_2$

fractional extraction of soybean deodorizer distillate using an extractor with a reboiler at the bottom and obtained 83.6% tocopherol recovery and an average concentration factor of 1.38 at the optimal conditions of 31.03 MPa, 363 K top and 343 K bottom temperature. Free fatty acids and squalene were concentrated in the extracts together with tocopherols whereas sterols were concentrated in the raffinates.

SCCO$_2$ column fractionation of soybean [41, 43, 45, 46] and rice bran oil [46] deodorizer distillates has been investigated to concentrate sterols and tocopherols. Brunner et al. [45] reported that the FFA were enriched in the top (in the extract), while the MG, tocopherols, and DG were enriched in the bottom (in the raffinate) fraction during fractionation of soybean oil deodorizer distillate. Saure and Brunner [43] achieved a tocopherol concentration of more than 70% using continuous column fractionation of soybean deodorizer distillate where squalene was almost completely found in the top product, whereas sterols and tocopherols were enriched in the bottom product. Dunford and King [46] developed a two-step column fractionation scheme (13.6 and 27.2 MPa at 313 K) for the enrichment of phytosterols from soybean and rice bran oil deodorizer distillates such that the FFA were removed in the first step and sterols were enriched in the oil fraction in the second step. Zhao et al. [41] used an extractor coupled to a fractionation column for the isolation of tocopherols and sterols from esterified soybean distillate. Sterols were left in the extractor whereas tocopherols were collected from the bottom of the column and FAME were collected in the separator.

SCCO$_2$ column fractionation has also been investigated for the production of squalene concentrates from olive oil deodorizer distillates [42, 44]. Bondioli et al. [42] used saponification and esterification steps to convert the FFA and FAE to TG in order to improve squalene separation prior to countercurrent continuous fractionation. Highest squalene purity and extraction yield was achieved at 15 MPa and 313 K, while a temperature gradient of 303-323 K along the column improved purity and yield. Ruivo et al. [101] studied countercurrent packed column fractionation of model mixtures containing 40% and 70% squalene and methyl oleate and achieved squalene yields as high as 90%.

SCF technology has also been investigated for the enrichment of minor oil components. SFE coupled with supercritical fluid chromatography (SFC) has been investigated for the enrichment of ferulate phytosterol esters from corn bran oil [102] and corn fiber oil [103] as well as tocopherols from soybean [104], wheat germ [104-106] and rice bran oils [104]. Enrichment of squalene by $SCCO_2$ extraction of olive husk oil [107] and column fractionation of shark liver oil [55, 56], as well as column fractionation of cod liver oil for the recovery of vitamin A [55] have also been studied. Ibanez et al. [62] investigated the use of column fractionation for the concentration of sterols and tocopherols from olive oil and concluded that $SCCO_2$ processing offers an alternative for the value added processing of low quality olive oil.

The use of fish oils as raw materials for the production of EPA and DHA concentrates has been widely investigated for nearly two decades [54, 58, 59, 61, 67]. As fractionation of TG is a rather complex and inefficient process, an esterification step usually precedes the fractionation where the TG are converted into more soluble FAE. While earlier studies used batch column fractionation to attain the concentration of EPA and DHA [54, 59], recent research is focused on the continuous column operation [58, 61].

Milk fat is a mixture of TG, which vary greatly in their physical properties such as melting point. Milk fat fractionation offers the potential of tailoring milk fat fractions for specific food applications, which would in turn increase milk fat utilization. $SCCO_2$ fractionation of milk fat has been investigated using fractional extraction with a density gradient [108, 109] and packed columns [63, 64] and resulted in the concentration of TG according to carbon number, yielding fractions with varying melting behavior. Column fractionation of milk fat has also been investigated for the concentration of conjugated linoleic acid to be used as a nutraceutical [66].

SCF technology is a "natural" and environmentally clean alternative for the processing of fats and oils, which offers the processor operational flexibility that can be exploited to meet the required objectives. A good understanding of the solubility behavior of lipids in multicomponent mixtures and solute properties are required to realize the full potential of SCF technology in fats and oils processing. Applications of SCFs have been widely investigated for the fractionation of lipid mixtures; however, studies on the fractionation of canola oil, the leading oil produced and refined in Canada, or its by-products are very scarce. The use of $SCCO_2$ in the processing of canola oil for fractionation purposes requires further attention with special focus on value-added processing.

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2. CORRELATING THE SOLUBILITY BEHAVIOR OF FATTY ACIDS, MONO, DI-, TRIGLYCERIDES AND FATTY ACID ESTERS IN SUPERCRITICAL CARBON DIOXIDE[1]

2.1. INTRODUCTION

Potential applications of supercritical fluid (SCF) technology in fats and oils processing have been extensively studied over the last two decades (Chapter 1). Although SCFs such as ethane [1-3] and propane [4] have been reported to be better solvents for lipids, the majority of the studies were carried out using supercritical carbon dioxide ($SCCO_2$), because it is a nonflammable and nontoxic solvent, which is inexpensive and readily available in high purity [5].

Knowledge of phase behavior is essential for the design of any SCF process. The lack of reliable phase equilibrium data has been one of the major impediments to the wider commercialization of SCF technology. The applicability of the existing database for lipids is further limited by the discrepancies between the literature data and the lack of phase behavior information, which is essential for accurate interpretation of the data.

Complete phase diagrams of binary systems of $SCCO_2$ and pure lipids are not available. Information on the melting behavior of lipids when in contact with $SCCO_2$ is quite limited. Most of the available information is based on the visual observation of the phases during phase equilibria studies and the indirect observation of a substantial increase in solubility in a certain temperature range due to the effect of phase change on solubility behavior. The physical properties of a substance are expected to have a substantial influence on the extraction process only in the narrow temperature range where the equilibrium shifts from solid-SCF to liquid-SCF [6].

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Increasing the hydrostatic pressure increases the melting point of the pure solid [7]. The phase behaviors of natural fats and oils, triolein (OOO), and trilaurin (LLL) from atmospheric pressure to 150 MPa [8] and of binary mixtures of *cis*-unsaturated fatty acids (FA) under various pressures up to 200 MPa [9] have been investigated to determine the effect of pressure on the phase behavior of lipid systems. However, when a solid is compressed in the presence of a SCF, the melting point of the solid decreases with increasing pressure because of the solubility of the supercritical solvent in the heavy liquid phase [10].

Bamberger et al. [11] studied the melting behavior of pure FA, triglycerides (TG) and mixtures of TG in CO_2 at 15 MPa and 313 K. They reported that lauric acid, myristic acid, LLL and trimyristin (MMM), LLL-MMM and LLL-MMM-tripalmitin (PPP) mixtures melted, while pure palmitic acid, PPP, LLL-PPP and MMM-PPP did not melt. Nilsson and Hudson [12] observed a melting point depression for PPP to 57°C at 17.2 MPa and concluded that at 40°C PPP would occur as a solid up to 31 MPa, but as a liquid at 60°C. In their study on the phase behavior of pure lipids, Hammam and Sivik [13] reported melting point depression for laurin glycerides, MMM, PPP and tristearin (SSS) in $SCCO_2$. The melting points of MMM, LLL, PPP and SSS at atmospheric pressure and the melting point depression in $SCCO_2$ determined by Hammam and Sivik [13] are given in Table 2.1.

Fats and oils are complex mixtures of various components that belong to different lipid classes including FA, monoglycerides (MG), diglycerides (DG), TG and fatty acid

Table 2.1. Atmospheric melting points and melting point depression of triglycerides in $SCCO_2$ [13].

Component	Formula	Molecular weight	Melting point (°C)[a]	Melting range in $SCCO_2$ (°C)[b]
Trilaurin	$C_{39}H_{74}O_6$	639.0	46.5	27-29
Trimyristin	$C_{45}H_{86}O_6$	723.2	58.5	38-42
Tripalmitin	$C_{51}H_{98}O_6$	807.3	66.4	48-51
Tristearin	$C_{57}H_{110}O_6$	891.5	73.5	58-61

[a] At 0.101325 MPa
[b] Up to 21 MPa

esters (FAE). In such multicomponent mixtures, complex intermolecular interactions lead to significant deviations from pure-component solubilities. Binary systems of various pure lipids and $SCCO_2$ have been studied by various researchers; however, multicomponent data are relatively scarce. An exhaustive literature survey of binary and multicomponent data of fish oil components was carried out by Staby and Mollerup [14]. Although limited, the information obtained by studying the extensive literature database for much simpler binary systems would be useful in the study of real mixtures. The general solubility trends of binary mixtures would contribute to our understanding of the fundamentals of lipid solubility in SCFs and, hence, provide a sound basis on which the solubility behavior of complex multicomponent mixtures can be evaluated. However, such a systematic study comparing the solubility behavior of the different lipid classes has not been carried out.

Therefore, the objectives of this study were: a) to compile a database of literature solubility data for pure components of FA, FAE, MG, DG and TG in $SCCO_2$, b) to correlate the lipid component solubility data using Chrastil's equation, and c) to establish the general trends in the solubility behavior of pure lipids of various classes in $SCCO_2$ as affected by operating conditions and solute properties. Chrastil's equation is an empirical model used quite commonly to correlate the solubility of lipid components. It was adopted in this study because it is an easy to use model, which does not require information on the properties of lipid components. Its parameters can be used to interpret the effect of operating conditions on solubility. It was used as a means to study the solubility behavior of lipid components in a systematic manner. Its value; however, is limited for predictive modeling of solubility data, which should involve in-depth thermodynamic modeling (eg. using an Equation of State (EOS) approach) of both phases present. In-depth thermodynamic modeling to describe solubility data was not attempted as it was outside the scope of this study.

2.2. SOLUBILITY DATA AND CORRELATION

2.2.1. Solubility data

The compilation of studies reporting the solubility of pure lipid components in $SCCO_2$ is presented in Table 2.2. As has been noted by others previously, there is discrepancy between the solubility data reported by various researchers, which limits the applicability of the existing database. Major factors contributing to such a discrepancy in the database are the purity of the samples used in the solubility measurements and the limitations of the experimental techniques used. The comparison of the literature data is further complicated by the necessity to use pure CO_2 density to convert the solubilities reported in different units such as mole fraction, weight fraction and weight percent to the same basis.

The solute purity is a critical factor because impurities present in the samples may affect the solubility. The solute solubility can be enhanced substantially in a

Table 2.2. Literature solubility data for binary systems of pure lipids and $SCCO_2$.

Component	No. of data [a]		Data presented as[b]	T (K)	P (MPa)	Purity[c] (%)	Experimental method[d]	Ref.
	liquid	vapor						
Fatty acids								
caproic acid	10	8	m.f.	313, 353	2.7-15.9	>95	R	15
lauric acid	-	4	w/w	308,318	13.9-26.9	99-100	D	16
	-	10	m.f.	313	7.7-24.8	99	D	11
	16	14	m.f.	333,353	2.6-27.7	>95	R	15
myristic acid	-	17	w/w	308-333	13.9-41.9	99-100	D	16
	-	7	m.f.	313	8.2-24.9	99.5	D	11
	-	6	m.f.	308	8.1-22.8	>99	D	17
	-	2	w/w	313,323	20	≥90	D	18
	-	-	q.s.	323	10-50	n.r.	Syn	19
palmitic acid	-	9	w/w	308-328	13.9-41.4	99-100	D	16
	-	9	m.f.	313	8.0-24.8	99-100	D	11
	-	6	m.f.	308	9.9-23.0	>99	D	17
	-	22	m.f. + w/v	298,313	8.0-18.7	>99	St	20
	-	6	w/w	308-323	20,30	≥96	D	18
	-	19	m.f.	318-338	14.2-57.5	95	D	21
	7	5	m.f.	353,373	13.6-30.5	>90	R	15
stearic acid	-	15	w/w	308-328	13.8-41.2	99-100	D	16
	-	8	m.f.	308	9.0-23.7	99[e]	D	22
	-	6	w/w	313-333	20,30	≥95	D	18
	-	7	w/v	313,333	10.1-25.3	n.r.	St	23
	-	18	m.f.	310,320	11.4-36.4	≥99.5	D	24
	-	17	m.f.	318-338	14.5-46.8	≥97	D	25
	-	g.	d.r. + r.s.	313	27.4-192.5	n.r.[f]	D	26
	-	9	m.f.	318	9.6-16.2	n.r.[f]	R	27
	-	11	m.f.	318	9.6-16.5	n.r.[f]	R	28
	-	6	m.f.	308	8-16	n.r.[f]	R	29
	-	9	m.f.	308	8-16	n.r.[f]	R	30
oleic acid	-	9	w / w	313-333	13.8-27.6	99-100	D	16
	-	12	m.f. + w/v	308,318	9.6-20.1	>99	D	31
	-	4	w/w	313,333	20,3	≥68	D	18
	12	12	m.f. + w/w	313,333	7.1-28.8	99	R	32
	-	9	w/v	313,333	10.1-25.3	n.r.	St	23
	-	20	w/v	303-323	10.6-27.9	n.r.	St	33
	15	12	w.f.	313-353	10.2-30.0	>90	R	34
	g.	g.	w.f.	313-353	2-20	70	St	35
	-	-	q.s.	323	10-50	n.r.	Syn	19
	-	15	m.f.	308,333	8.5-19.1	>90	R	36
	-	10	w%	323,333	12.4-20.6	≥99	D	37
	17	17	m.f.	313,333	3.4-31.1	99	R	38
	16	-	m.f.	313,323	0.9-8.0	90	R	39
linoleic acid	-	9	w/w	313-333	13.8-27.6	99-100	D	16
	12	12	m.f. + w/w	313,333	6.3-27.1	99	R	32
behenic acid	-	9	w/v	313,333	8.1-25.3	n.r.	St	23
	-	12	m.f.	308,318	8-16	n.r.[f]	R	40
Monoglycerides								
monolaurin	-	6	m.f.	313,338	15-40	99	D	41
	-	g.	w/v	313	15-35	99	D	42

Table 2.2. continued

Component	No. of data[a]		Data presented as[b]	T (K)	P (MPa)	Purity[c] (%)	Experimental method[d]	Ref.
	liquid	vapor						
	-	-	q.s.	323	10-50	n.r.	Syn	19
monostearate	-	-	q.s.	323	10-50	n.r.	Syn	19
monoolein	-	12	m.f.	308,333	10.4-18.9	97	R	36
	-	11	w%	323,333	15.1-30.9	≥99	D	37
Diglycerides								
dilaurin	-	9	m.f.	313,338	15-40	99	D	41
	-	g.	w/v	313	15-35	99	D	42
	-	-	q.s.	323	10-50	n.r.	Syn	19
diolein	-	11	w%	323,333	15.1-30.9	≥99	D	37
Triglycerides								
tributyrin	-	8	w/v	313,333	10.1-25.3	n.r.	St	23
	-	g.	w/v	313	8-35	99	D	42
tricaproin	-	g.	w/v	313	15-35	99	D	42
tricaprylin	10	10	m.f.	333,353	5.3-25.0	>90	R	15
	42	29	w%	313-393	5.0-32.2	99	St	43
	-	g.	w/v	313	10-35	99	D	42
tricaprin	-	g.	w/v	313	15-35	99	D	42
trilaurin	-	6	m.f	313	9.1-25.3	99	D	11
	5	4	m.f.	353	10.3-31.8	>90	R	15
	-	16	m.f.	308-333	15-40	99	D	41
	-	g.	w/v	308-333	15-35	99	D	42
	-	g.	m.f.	308-328	8.5-36.2	n.r.	D	44
trimyristin	-	8	m.f	313	9.5-30.4	99[e]	D	11
	-	g.	m.f.	308-328	8.5-36.2	n.r.	D	44
tripalmitin	-	7	m.f	313	12.2-29.7	99[e]	D	11
	-	18	w/v	313-353	8.1-25.3	n.r.	St	23
	5	5	m.f.	353	5.6-24.3	>90	R	15
	-	16	m.f. + w/v	298,313	8.6-18.2	>96.5	St	20
	-	9	w/w	313,333	17.2-31.0	n.r.	D	12
	-	g.	m.f.	308-328	8.5-36.2	n.r.	D	44
	9	9	mf	333, 353	10-50	95	St	45
tristearin	-	6	w/w	313-333	20,30	≥65	D	18
	-	11	w/v	313,333	8.1-25.3	n.r.	St	23
	-	g.	w% vs vol	313,333	9.8-27.0	n.r.	D	46
	-	g.	m.f.	308-328	-	n.r.	D	44
	9	9	mf	333, 353	20-50	9	St	45
triolein	-	4	w/w	313,333	20,30	≥65	D	18
	-	20	w/v	313-353	8.1-25.3	n.r.	St	23
	8	8	w.f.	313,333	15.3-31.0	>70[e]	R	34
	-	10	w/v	308	9.6-22.0	99	St	47
	-	11	w%	323,333	17.2-30.9	≥99	D	37
	-	5	w/w	313	17.2-31.0	n.r.	D	12
	-	113	w/w	308-328	8.0-21.0	99	St	48
	-	g.	w% vs vol	313	19.6	n.r.	D	46
	-	g.	w.f.	298-333	7-20	98	R	49
	8	8	mf	333, 353	20-50	95	St	45
trilinolein	-	8	w/v	313,333	8.1-25.3	n.r.	St	23
Methyl esters								
lauric acid	25	12	m.f.	313-333	2-12	98	R	50
	-	g.	m.f.	313,333	6.5-20	n.r.	D	51
myristic acid	43	14	m.f.	313-333	0.88-12	>99	R	39

Table 2.2. continued

Component	No. of data [a]		Data presented as[b]	T (K)	P (MPa)	Purity[c] (%)	Experimental method[d]	Ref.
	liquid	vapor						
	24	16	m.f.	313-343	1.16-15.97	95	R	52
	-	g.	m.f.	313,333	6.5-20	n.r.	D	51
palmitic acid	38	16	m.f.	313-333	0.98-13	95	R	39
	38	19	m.f.	313-343	1.01-18.29	95	R	52
stearic acid	27	24	m.f.	313-343	2.15-20.42	98	R	52
	-	g.	m.f.	313,333	6.5-20	n.r.	D	51
oleic acid	-	8	w%	323,333	11.0-15.1	≥99	D	37
	13	13	m.f + w/w	313,333	4.1-19.0	99	R	32
	38	29	m.f.	313-343	1.8-20.0	72	R	52
	8	8	m.f.	313,333	2.9-13.7	99	R	38
	-	g.	m.f.	313,333	6.5-20	n.r.	D	51
	19	22	m.f.	313,343	2.0-19.7	98-99	R	53
linoleic acid	13	13	m.f. + w/w	313,333	3.8-20.3	99	R	32
	8	9	m.f.	313,343	4.1-21.3	99	R	54
	-	g.	m.f.	313,333	6.5-20	n.r.	D	51
	7	7	m.f.	343	2.1-14.0	99	R	55
Ethyl esters								
lauric acid	-	2	q.s.	298,305	17.2	n.r.	St	6
palmitic acid	-	g.	w / v	298-328	6.9-17.2	>99	D	56
stearic acid	29	37	m.f.	313-333	1.5-18.3	>90	R	57
oleic acid	38	40	m.f.	313-333	1.1-18.6	>90	R	57
	-	g.	m.f.	313-373	9-25	99	D	58
	-	g.	w / v	298-328	6.9-17.2	>99	D	56
linoleic acid	33	35	m.f.	313-333	2.0-17.0	>90	R	57
eicosatrienoic acid	-	g.	m.f.	313-373	9-25	99	D	58
arachidonic acid	-	g.	m.f.	313-373	9-25	99	D	58
EPA	36	26	m.f.	313-333	2.0-20.0	>90	R	57
	-	g.	w/v	298-328	6.9-17.2	90.9	D	56
DHA	28	27	m.f.	313-333	1.9-21.1	>90	R	57
	-	g.	m.f.	313-373	9-25	99	D	58
	-	g.	w/v	298-328	6.9-17.2	90.3	D	56

[a] No of data: g., data presented only in graphs

[b] Data presented as: m.f., mole fraction; w.f., weight fraction; q.s., quantitative solubility; d.r., detector response; r.s., relative solubility; w% vs vol., graph of w% extracted versus volume of CO_2 consumed

[c] Purity: n.r., not reported

[d] Experimental method: R, recirculation; D, dynamic; St, static; Syn, synthetic

[e] Purified further by supercritical fluid extraction

[f] Purified further by recrystallization

multicomponent system because of the presence of other solutes [11, 59, 60]. On the other hand, the solubility of an individual component may decrease with the addition of a second solute if the solute mixture is a liquid under extraction conditions [61]. The solubilities of samples with different purities have been compared in order to demonstrate the effect of the sample purity on the solubility [11, 37]. Bamberger et al. [11] compared the solubility of 90% PPP with that of 99% pure PPP under the same conditions and reported more than one order of magnitude higher solubility for the lower purity sample. As well, Nilsson et al. [37] reported that the solubility of 65% OOO was nearly twice that of the 99% purity sample. The overall effect of impurities on the solubility of a sample will depend on the nature of the solute and impurities as well as the level of impurities.

2.2.2. Experimental techniques

The experimental techniques used in the investigation of high-pressure phase equilibria have been described and reviewed by various authors [62-66]. Analytical techniques involve compositional analysis by sampling once equilibrium is attained. In synthetic methods, a premixed sample of known composition is charged to the equilibrium cell for determination of phase equilibria without sampling. Analytical methods are subdivided into three types: static, dynamic and recirculation depending on how the equilibrium is attained. Static techniques involve loading the solute and solvent into a high-pressure cell and allowing them to reach equilibrium. A thorough agitation is applied by stirring or rocking to ensure a rapid approach to equilibrium. Dynamic methods are characterized by the passage of a gas through a layer of liquid to achieve the saturation of the exit gas phase with the liquid-phase components. In recirculation methods, one or both of the phases are recirculated to achieve equilibrium conditions faster.

A major limitation of the experimental apparatus used for solubility measurements in many studies is the lack of a visual observation window on the high-pressure cell due to its high cost, which may lead to an error in solubility measurements.

At high pressures, the density of the SCF-rich phase can become greater than that of the solute-rich phase. This phenomenon is referred to as density inversion and results in the liquid phase to be pushed out of the cell, leading to erroneous solubility information [7]. If the experimental system used does not allow the visual observation of a potential density inversion, the solubility data cannot be interpreted accurately. For example, Maheshwari et al. [16] observed very high solubilities for lauric acid at 34.2 MPa/318 K and 40.6 MPa/308 K as well as for myristic acid at 41.9 MPa/318 K; however, they could not ascertain whether these results were due to the proximity of experimental conditions to the upper critical end point or density inversion. Another aspect that cannot be observed without a window on the cell is the phase equilibria, and it is not possible to ascertain the physical state of the solute from the shape of the solubility isotherms. Therefore, it is essential to have information on the melting behavior of the solid solutes in order to interpret the solubility data accurately [10].

Other factors that have been cited by various authors to explain the discrepancy between literature data are the entrainment of a liquid solute while sampling and failure to achieve equilibrium in the system. Moreover, greater solubility of solutes at higher temperatures, which results from the nonretrograde solubility behavior, can result in erroneously low data due to solute hold-up or in complete clogging of the tubing between the extractor and metering valve [12, 16].

2.2.3. Correlation of solubility data

Chrastil's equation [23] was used to describe the solubility data compiled for various pure lipid components of different classes. This model is based on the formation of a solute-solvent complex upon association of the solute and solvent molecules.

Chrastil's equation establishes a linear relationship between ln(solubility) and ln(density), which was first observed by Stahl et al. [67] as follows:

$$\ln c = k \ln d + a/T + b \tag{2.1}$$

where c is the solubility of the solute in the supercritical solvent (g/L), d is the density of the pure solvent (g/L) and k (association number) is the number of molecules in the solute-solvent complex. Parameter a is dependent on the total heat of the reaction (heat of solvation + heat of vaporization), and b is dependent on the molecular weights of the solute and solvent and the association constant. Parameter k, which is the slope of the solubility isotherm, reflects the density dependence of solubility. Parameter a, which is the slope of ln(solubility) versus 1/T plot, is a measure of the temperature dependence of solubility at constant density.

The concentration and temperature range of the model is determined by the validity range of its approximations. Deviation from a linear relation is expected at high solute concentrations as the solvent density starts to deviate from the density of the pure supercritical solvent [23]. The solute polarity has also been reported to affect the accuracy of the model. The model was not accurate for highly polar solid solutes up to a reduced density of 1.5, indicating density dependence of the association number [68].

In this study, model parameters a, b and k were estimated from the lipid solubility data using multivariate regression analysis of the SAS statistical software package [69]. To improve the goodness of fit of the model, solubilities higher than 0.1 kg/kg [16] and data for solutes with unknown purities or with purities <95% were excluded from the analysis. Data that consistently deviated from the rest of the database [18, 32, 48, 53, 55] were also excluded. CO_2 density data used in the model as well as for the conversion of solubility data to the required units (g/L) were obtained using the pressure/density calculator in Dionex SFC/GC Control Software (version 3.32, Mississauga, ON).

The expected linear relationship between ln(solubility) and ln(density) has been observed for FA [23, 31, 33], TG [23,48] and fatty acid ethyl esters [56, 58] by others; however, the temperature dependence of k has not been well established. Both parallel and non-parallel isotherms have been reported for pure lipids. Some researchers correlated experimental data at only one temperature [70] or did not provide a comparison of the k values at different temperatures [16]. In this study, both the overall k values (using eq 2.1) valid for the whole temperature range studied and the individual k values at each temperature (using eq 2.2) were estimated in order to determine the temperature dependence of k and any associated trend. At constant temperature, eq 2.1 simplifies to

$$\ln c = k' \ln d + b' \tag{2.2}$$

2.3. RESULTS AND DISCUSSION

2.3.1. Model parameters

2.3.1.1. Regression analysis using equation 2.2

The model parameters estimated using eq 2.2 at each temperature are presented in Table 2.3. Only temperatures at which significant parameters ($p \leq 0.05$) could be estimated by the regression analysis were reported. When data from two studies were included in the analysis, the discrepancies between some studies were reflected in low R^2 values. The results of the analysis of individual and combined data sets for palmitic acid at 313 K, stearic acid at 328 K, monoolein (MO) at 333 K, and PPP at 313 K are given in Table 2.4

Table 2.3. Model parameters for pure lipids estimated using equation 2.2 (ln c = k' ln d + b').

Solute	T (K)	k ± standard error	b±standard error	R^2	Data from ref.
Fatty acids					
caproic acid	353	4.6±0.6	-25.0±3.4	0.967	15
lauric acid	313	7.8±0.2	-48.2±1.1	0.999	11
lauric acid	333	9.7±0.4	-60.4±2.6	0.997	15
lauric acid	353	9.1±0.1	-55.5±0.3	1.000	15
myristic acid	308	4.9±0.3	-30.2±2.0	0.974	16, 17
myristic acid	313	7.2±0.3	-45.1±2.0	0.987	11, 16
myristic acid	323	5.5±0.4	-33.1±2.4	0.996	16
palmitic acid	308	5.3±0.5	-35.3±3.3	0.944	16, 17
palmitic acid	313	6.8±0.7	-43.8±4.3	0.873	11, 20
palmitic acid	318	7.3±1.2	46.3±8.1	0.859	16, 21
palmitic acid	328	9.5±0.9	-60.1±6.1	0.947	16, 21
palmitic acid	338	8.0±0.7	-49.5±4.5	0.985	21
stearic acid	308	5.0±0.9	-34.5±5.0	0.762	16, 22
stearic acid	310	2.3±0.5	-15.5±3.6	0.834	24
stearic acid	320	4.7±0.4	-30.1±2.7	0.956	24
stearic acid	328	7.0±0.9	-44.0±5.9	0.900	16, 25
stearic acid	338	8.2±0.3	-51.7±2.2	0.995	25
oleic acid	308	8.8±0.3	-56.9±2.2	0.995	31
oleic acid	313	8.8±0.6	-56.3±4.3	0.959	16, 38
oleic acid	318	6.8±0.4	-42.7±2.4	0.988	31
oleic acid	323	9.1±0.5	-58.1±3.0	0.985	16, 37
oleic acid	333	7.9±0.8	-50.0±4.9	0.900	16, 38
Monoglycerides					
monolaurin	313	5.3±0.4	-34.2±2.9	0.981	41
monoolein	308	8.1±1.2	-54.2±8.2	0.896	36
monoolein	323	7.7±0.4	-49.8±2.4	0.993	37
monoolein	333	10.4±1.6	-68.1±10.4	0.827	37, 36
Diglycerides					
dilaurin	313	7.4±0.5	-46.6±3.5	0.981	41
diolein	323	10.7±0.4	-70.1±2.4	0.997	37
diolein	333	10.4±0.4	-67.6±2.3	0.995	37
Triglycerides					
tricaprylin	313	4.1± 0.4	-23.9±2.5	0.923	43
tricaprylin	353	10.2 ± 0.6	-62.0±3.8	0.980	43
tricaprylin	393	7.8 ± 0.6	-46.2±3.5	0.984	43
trilaurin	313	11.3 ± 0.9	-73.1±5.7	0.966	11, 41
trimyristin	313	9.3 ± 0.4	-61.0±2.5	0.991	11

Table 2.3. continued

Solute	T (K)	k ± standard error	b±standard error	R^2	Data from ref.
tripalmitin	313	4.5 ± 1.2	-32.1±8.1	0.601	11, 20
tripalmitin	333	9.3±1.2	-60.3±7.9	0.970	45
tripalmitin	353	10.7±0.6	-69.2±4.2	0.993	45
triolein	308	8.4 ± 0.8	-55.4±5.5	0.928	49
triolein	323	12.9 ± 0.6	-85.4±4.1	0.993	37
triolein	333	11.9 ± 0.5	-78.7±3.5	0.985	37, 46
triolein	353	8.2±1.5	-53.7±10.2	0.933	45
Methyl esters					
lauric acid	333^a	4.7±0.4	-26.3±2.0	0.983	50
myristic acid	313^a	2.0±0.4	-10.6±2.2	0.897	39, 52
myristic acid	333^a	2.1±0.3	-11.2±1.6	0.919	39, 52
palmitic acid	333^a	2.6±0.5	-14.4±2.7	0.910	39, 52
palmitic acid	333	7.8±1.2	-46.2±7.3	0.958	39, 52
stearic acid	313	7.5±0.3	-45.4±2.2	0.992	52
stearic acid	343	11.4±0.4	-69.6±2.4	0.998	52
oleic acid	323	7.7±0.0	-46.5±0.2	1.000	37, 38
linoleic acid	343	10.9±0.5	-66.9±3.4	0.998	54
Ethyl esters					
stearic acid	313	6.1±1.1	-36.4±7.1	0.938	57
stearic acid	323	5.3±0.8	-31.3±5.0	0.940	57
stearic acid	333	5.9±1.2	-34.5±7.7	0.887	57
oleic acid	313	7.6±0.7	-46.2±4.4	0.984	57
oleic acid	323	8.4±0.3	-50.8±1.8	0.997	57
oleic acid	333	7.3±0.8	-43.6±4.9	0.968	57
linoleic acid	313	7.1±0.7	-43.0±4.3	0.974	57
linoleic acid	323	6.8±1.0	-41.0±6.2	0.962	57
linoleic acid	333	7.5±2.2	-44.9±14.3^b	0.791	57
EPA	313	8.5±0.2	-52.4±1.6	0.997	57
EPA	323	9.7±0.5	-60.1±3.1	0.993	57
EPA	333	8.5±0.2	-52.1±1.2	0.998	57
DHA	313	6.8±0.5	-41.5±3.5	0.969	57
DHA	323	8.2±0.5	-50.4±3.3	0.977	57
DHA	333	8.6±0.4	-53.1±2.6	0.985	57

[a] Parameters estimated for d<470 g/L.

[b] Not significant ($p>0.05$)

Table 2.4. Discrepancies in the results of different studies included in correlation using equation 2.2 (ln c = k' ln d + b').

Solute	T (K)	k' (R^2)	Ref.	k' (R^2)	Ref.	Combined data set k' (R^2)
palmitic acid	313	3.7 (0.985)	20	7.8 (0.997)	11	6.8 (0.873)
stearic acid	328	5.0 (0.998)	16	8.7 (0.955)	25	7.0 (0.900)
monoolein	333	7.5 (0.997)	37	3.7 (0.808)	36	10.4 (0.827)
tripalmitin	313	1.4 (0.969)	20	11.1 (0.991)	11	4.5 (0.601)

to illustrate the extent of variation between studies. Due to the low R^2 values, these temperatures were not taken into consideration while interpreting the results because it was not possible to ascertain the source of the discrepancy.

Preliminary analyses of the solubility data using Chrastil's equation showed a deviation from linearity at low solvent densities (280-400 g/L) for lauric acid at 313-353 K and oleic acid at 333 K. The exclusion of low-density data improved the R^2 values considerably (from 0.736 to 1.000 for lauric acid at 353 K) but did not improve the correlation for the other FA. Therefore, a low-density limit could not be determined and low-density data were only excluded for lauric and oleic acids. Lowest density data (223 g/L) was also excluded for PPP at 353 K.

The slopes of the solubility isotherms (k' values) were observed to be dependent on the physical state of the solute. At 308 K, myristic, palmitic, and stearic acids are solid and have similar k' values in the range of 4.9-5.3 (Table 2.3). An increase in the k' value was observed from 308 to 313 K for myristic acid, 308 to 328 K for palmitic acid, and from 320 to 338 K for stearic acid. This increase is most probably caused by the melting of these solutes in this temperature range due to the solvent effect of $SCCO_2$. The low k' values for solid FA were accompanied by relatively low R^2 values due to deviation from linearity at high densities as the solubility approached a maximum. The density dependence of solubility decreased for these compounds at high solvent densities. No clear trend with temperature could be observed in the k' values of liquid FA. The k' values for TG, all of which were liquid over the studied temperature range, were in the range of 4.1-12.9. In general, the association constants for TG appeared to be higher than those for the corresponding FA, indicating greater association between CO_2 and nonpolar TG than between CO_2 and polar FA.

When the ln(c) vs ln(d) plots of FAE were analyzed, there appeared to be a break in the solubility isotherms of all the esters around the critical density. Because it was not possible to determine the density limit accurately, the critical density of CO_2 (470 g/L) was used as the density limit for the model for consistency in analysis. The high-density and low-density data were analyzed separately, and two sets of model parameters were reported whenever there were enough data for the analysis. The k' values for methyl esters were in the range of 2.0-4.7 for low-density ($d<470$ g/L) and 7.5-11.4 for the high-density ($d>470$ g/L) range (Table 2.3). Although deviation around critical conditions has been noted for highly polar solid solutes in $SCCO_2$ [39], such a deviation has not been reported for pure lipids. On the contrary, Liong et al. [58] reported excellent correlation for ethyl esters ($R^2 \geq 0.994$) at 313-373 K and 9-25 MPa. This break in the isotherms might be due to model limitations or due to the proximity to the critical point ($d<470$ g/L) resulting in higher variation within the experimental data as operating conditions have a more pronounced effect on solubility in this region. Lower R^2 values of some esters could be due to the dependence of limit density on solute properties among esters. The k' values for methyl esters could only be compared at 343 K for methyl stearate and methyl linoleate and were found to be similar. No trend could be observed in the k' values of ethyl esters with temperature, which were in the range of 5.3-9.7. The k' values for stearic acid ethyl ester were lower than that for the other ethyl esters.

The k' values and, hence the density dependence of the solubility, were observed to be strongly dependent on the physical state of the solute. Therefore, regression analysis using eq 2.2 should be carried out at each temperature for solutes for which a phase transition is anticipated over the experimental range to correlate experimental solubility data. For the liquid solutes, no general trend could be observed in the k' values with temperature. It is necessary to carry out the regression analysis at each temperature (eq 2.2) to ascertain the parallel nature of the isotherms before estimating common slopes using eq 2.1 for these compounds. Because deviations from linearity were observed to be density dependent, experimental data over similar density ranges should be used in the analysis. The model parameters listed in Table 2.3 can be used to provide preliminary information on the solubility of pure lipids in $SCCO_2$ at constant temperature.

2.3.1.2. Regression analysis using equation 2.1

Table 2.5 gives the model parameters estimated using eq 2.1. The a values for all of the lipids studied had negative values (Table 2.5), indicating an inverse relationship between ln(c) and 1/T or an increase in the solubility with temperature at constant density. Therefore, the absolute values were considered while comparing the a values.

In the FA series, the highest melting FA, stearic acid, had the highest a value, indicating the highest temperature dependence of solubility at constant density, whereas the lowest value was obtained for lauric acid. FAE and TG had lower a values than FA (-2473 to -1448). Increasing temperature at constant density results in an increase in the solubility of the solute due to an increase in its vapor pressure. Thus, the a value is

Table 2.5. Model parameters for pure lipids estimated using equation 2.1 ($\ln c = k \ln d + a/T + b$).

Solute	**k ± standard error**	**a ± standard error**	**b ± standard error**	**R^2**
Fatty acids				
lauric acid	8.0±0.3	-2853±507	-40.7±2.3	0.991
myristic acid	6.4±0.3	-9300±1727	-10.2 ±5.9[a]	0.958
palmitic acid	7.0±0.4	-12029±1043	-7.0±4.1[a]	0.909
stearic acid	5.8±0.5	-15890±741	12.0±3.7	0.933
oleic acid	7.9±0.4	-3982±691	-38.1±2.3	0.922
linoleic acid	9.7±0.9	-5211±1626	-46.3±5.3	0.954
Monoglyceride				
monoolein	10.7±1.1	-7925±1360	-45.8±6.1	0.828
Diglyceride				
diolein	10.5±0.3	-4601±533	-54.3±1.9	0.996
Triglycerides				
tricaprylin	5.3±0.5	-355±634[a]	-30.3±3.2	0.848
triolein	10.1±0.6	-1448±401	-62.2±4.0	0.922
Ethyl esters				
stearic acid	5.8±0.5	-2446±857	-26.7±3.9	0.926
oleic acid	7.8±0.3	-1947±503	-40.9±2.7	0.980
linoleic acid	7.2±0.6	-2193±896	-36.2±4.4	0.921
EPA	8.6±0.2	-2473±262	-45.2±1.2	0.994
DHA	7.8±0.3	-1784±529	-42.1±2.5	0.966
Methyl ester				
stearic acid	8.0±0.6	-2099±479	-41.9±3.5	0.968

[a] Not significant (p>0.05)

indicative of the relative effect of temperature on the vapor pressure of solutes. Latent heat of vaporization of a solute is proportional to the slope of the log of vapor pressure versus 1/T plot and therefore is indicative of the temperature dependence of vapor pressure. Heat of vaporization data for lipids are relatively sparse, available data indicates an order of FA>FAME>TG and an increasing trend with carbon number in a homologous (TG) series [71, 72]. Lower a values observed for FAE and OOO are thus consistent with their lower heat of vaporization values. No trend could be observed for the b values, which were in the range of -62.2 to +12.0. The estimated model parameters (Table 2.5) can be used to calculate the lipid solubility for those solutes that have been shown to have a common slope over the range of interest.

2.3.2. Solubility behavior

The literature data were reviewed in order to establish the general trends of the solubility behavior of pure lipids in $SCCO_2$. To observe the effect of operating conditions and solute properties on the solubility, the data were analyzed in solubility versus pressure and solubility versus density plots. The model parameters were used to compare the solubility isotherms for analogous glyceride series.

The differences in solute properties such as molecular weight, polarity, and vapor pressure should be considered while comparing the solubility behavior of lipid classes. Physical properties of major lipid components have been discussed in Chapter 1 (Section 1.2.1, Table 1.2).

As expected, an isothermal increase in the pressure increased the solubility because of an increase in the solvent density and, hence, the solvation power. The pressure effect was considerable in the vicinity of the critical density, because the solvent density is very sensitive to changes in the pressure in this region. A comparison of solubility isotherms for several FA at 308 K is given in Figure 2.1. At this temperature myristic, palmitic, and stearic acids are solid, whereas oleic acid is liquid over the entire pressure range. Figure 2.2 is a plot of the FA solubility isotherms under experimental conditions where all the solutes are liquid. Because FA solubility data at 338 K were not available for all the FA, 313 K data were used for myristic, lauric and oleic acids, which were liquid at that temperature.

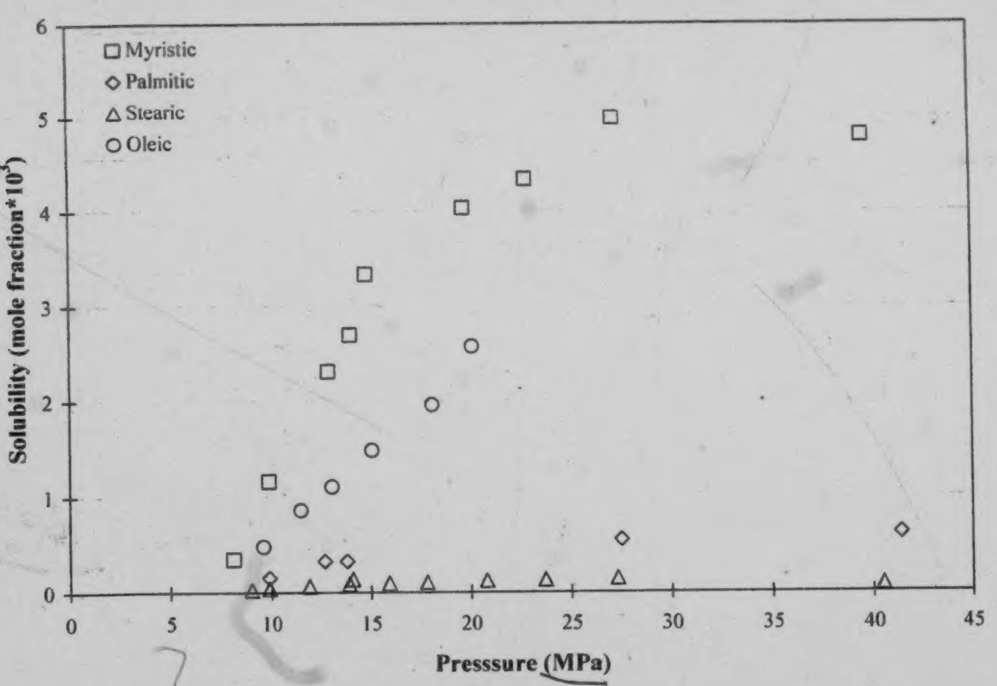

Figure 2.1. Solubility isotherms of myristic, palmitic, stearic, and oleic acids in $SCCO_2$ at 308 K.

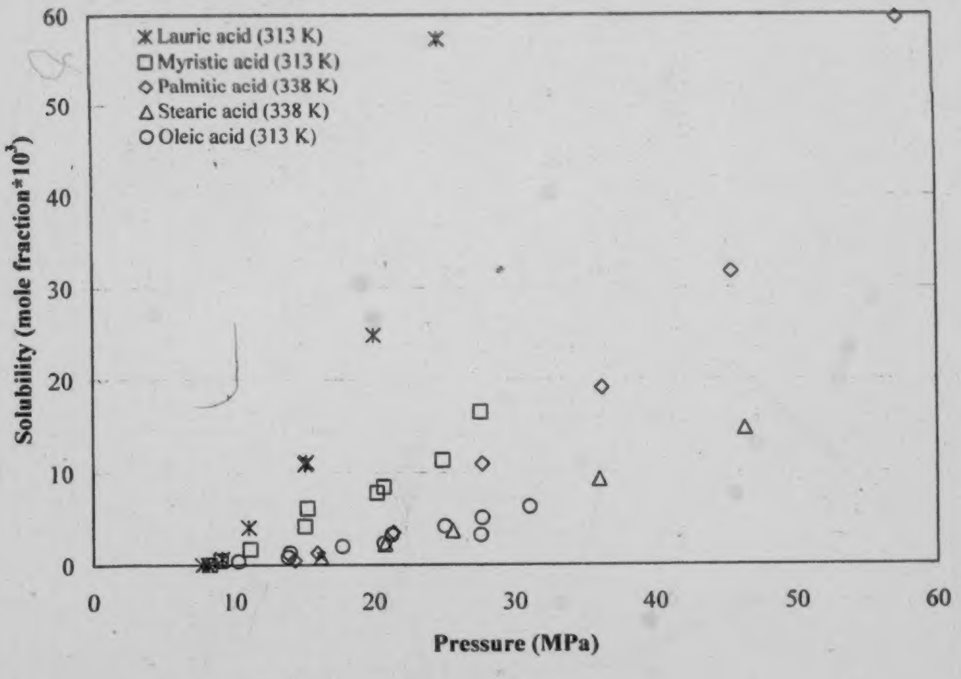

Figure 2.2. Solubility isotherms of lauric, myristic, palmitic and oleic acids at 313 K and stearic and palmitic acids at 338 K in $SCCO_2$.

The differences in the pressure dependence of lipid solubility reflect the changes in phase behavior due to phase transition of a solute or differences between the phase behavior of different solutes. The pressure dependence of the lipid solubility isotherms was dependent on the volatility and physical state of the solute. FAE, which were the most volatile of the compounds studied, had the highest pressure dependence. While liquid or liquefied solutes showed increasing solubility with pressure over the entire experimental range (Fig. 2.2), a solubility maximum was approached in the isotherms of solid solutes (Fig. 2.1). The solubility of stearic acid was observed to decrease with pressure at pressures ≥30 MPa and temperatures up to 318 K. A solubility maximum was first observed for stearic acid by Czubryt et al. [26], around 30 MPa at 313 K. Kramer and Thodos [25] also observed maximum solubility for stearic acid at pressures around 28-30 MPa at 318 K.

In a homologous series, the solubility trends followed the trends in vapor pressure such that solubility decreased with increasing carbon number/molecular weight (Figs. 2.1 and 2.2). Unsaturation had a significant effect on solubility behavior only when it led to a difference in physical state of the solutes as observed for oleic and stearic acids. Liquid oleic acid was more soluble than solid stearic acid at 308 K (Fig. 2.1), whereas the solubilities were fairly similar when both solutes were liquid (Fig. 2.2). In the unsaturated acid or ester series, solubility changed only slightly with degree of unsaturation. The solubilities of FAE with a higher degree of unsaturation were reported to be both higher [57] and lower [51, 58] than those with less unsaturation by different researchers. For the esters, which were the most soluble lipid class, the effect of molecular weight/carbon number was more pronounced than the effect of unsaturation. Methyl esters had higher solubilities than the corresponding ethyl esters due to their higher vapor pressures.

Increasing the temperature at constant CO_2 density increases the solubility because of an exponential increase in the solute vapor pressure. Temperature effect at constant CO_2 density was observed for all compounds, although the magnitude of the effect varied (Table 2.5).

An isobaric increase in the temperature decreases the solvent density and increases the vapor pressure of the solute. The overall impact of these two competing effects is dependent on the pressure. Below the crossover pressure, the density effect predominates and the solubility decreases with increasing temperature, which is referred to as retrograde behavior. Above this crossover point, the solubility increases with temperature because of the vapor pressure effect [60]. This crossover is expected to occur at higher pressures for solutes whose vapor pressures and solubilities are only slightly affected by temperature [73].

The existence of a crossover pressure in solid-SCF systems has been suggested as an indication of the reliability and consistency of experimental solubility data [74]. For solid lipid-SCF systems where one of the operating temperatures exceeded th[illegible]elting point of the lipid, solid- and liquid-phase isotherms were analyzed separately because a common crossover pressure could not be observed for such systems [74]. However, the interpretation of the data was hindered by the lack of reliable phase behavior information for these systems and the variability between literature data. Although distinct crossover pressures could not be observed for all lipids, some general trends could be reached in this study.

The solubility of lauric acid and FAE showed retrograde solubility behavior over the entire experimental range. A crossover in solubility isotherms (333-353 K) was observed for SSS at 30-40 MPa. A crossover pressure of 31 MPa has been observed for OOO solubility isotherms (313–333 K) [37]. However, solubility data of Weber et al. [45] showed retrograde solubility behavior for OOO in the pressure range 20-50 MPa and 333-353 K. This is an unexpected observation as nonretrograde solubility has been well documented for vegetable oils (which are comprised of 90-99% TG) at pressures higher than 30 MPa [75, 76].

It is important to differentiate between nonretrograde solubility behavior due to the competing effects of temperature on density and vapor pressure and solubility increase with temperature due to phase change. In order to determine the effect of temperature on solubility it is necessary to compare isotherms representing the same phase. For example, Nilsson et al. [12] compared PPP data at 313 K where PPP is solid and 333 K where it is liquid and concluded that PPP showed nonretrograde behavior. However, solubility of liquid PPP (333-353 K) decreased with increasing temperature in the pressure range 20-50 MPa. Therefore, when reporting the effect of temperature of solubility it is necessary to note the physical state of the substance and any phase change that might occur in the experimental range. Temperature effect in the solid region could not be determined for PPP as only one temperature (313 K) was studied.

Solid FA were in the nonretrograde region over the entire experimental range, indicating that the crossover pressures were lower than or close to the minimum pressure used. There appeared to be a second crossover pressure in the region where these solutes liquefy, which was considerably higher than the value for the solid solutes. The crossover pressures for palmitic and stearic acids under experimental conditions where they were liquid were around 20-25 MPa. Crossover pressures around 25 MPa were observed for dilaurin and trilaurin [41]. There appeared to be a crossover around 28 MPa for MO and diolein (DO) [37].

These findings are consistent with the trends observed in a values and indicate a shift in the crossover of isotherms to higher pressures with decreasing temperature dependence of solubility at constant density. Although it has been noted that a common crossover pressure could not be observed for solid-SCF systems where one of the operating temperatures exceeded the melting point of the solid [74], the existence of two distinct crossover pressures for different phases of the same substance has not been demonstrated. Further research is needed to determine the effect of a phase change on the crossover behavior hence temperature dependence of solubility for lipids.

When the solubilities in a glyceride series (MG, DG, TG) are compared, differences in the polarity of the solutes as well as their vapor pressure should be taken into account. In both olein and laurin glycerides, FA were the most soluble compounds over the entire experimental range. Different k' values of the laurin glycerides led to crossover of the solubility isotherms of LLL and dilaurin; and monolaurin and LLL at two different points (Fig. 2.3). For dilaurin and LLL, the crossover density was calculated to be 870 g/L. At densities >870 g/L, LLL, which has a lower vapor pressure, was more soluble than the more polar dilaurin. However, below this pressure, dilaurin was more soluble than trilaurin. Crossover density for monolaurin and LLL was determined to be 611 g/L (Fig. 2.3). While interpreting the laurin glycerides plot, it must be noted that lauric acid, dilaurin, and LLL were liquid, whereas monolaurin was solid at 313 K. The

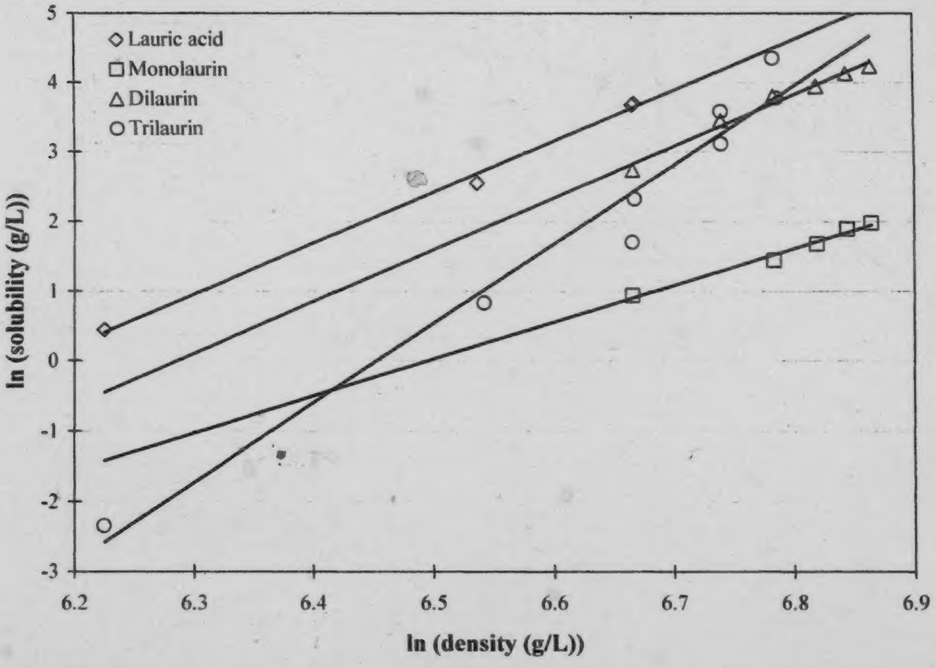

Figure 2.3. Solubility isotherms for laurin glycerides in $SCCO_2$ at 313 K (Solid lines represent regression results obtained using equation 2.2).

low solubility of monolaurin was in part due to the physical state of the solute. For the olein glycerides (Fig. 2.4), where all of the solutes were liquid under the experimental range, the least soluble compound was OOO. A crossover of the solubility isotherms of DO and MO was observed at a density of 794 g/L. At densities >794 g/L, the less polar DO was more soluble. Solubility in $SCCO_2$ is affected by vapor pressure of the solute and intermolecular interactions. The relative contribution of these effects is dependent on solvent density and hence operating conditions as highlighted by these observations. At lower solvent densities, relative solubility is dictated by vapor pressure, whereas the effect of solute polarity and hence intermolecular interactions dominates at higher densities.

In general, the solubilities of FAs were always higher than those of the remaining glycerides for all of the systems studied. However, there can be a crossover between FA and glycerides of different series because of the competing effects of polarity and vapor pressure on the solubility isotherms as noted by Bamberger [11] for LLL and palmitic acid at 313 K.

2.4. SUMMARY AND IMPLICATIONS

Development and potential commercialization of $SCCO_2$ technology in fats and oils processing require reliable and sufficient design data and a good understanding of the solubility behavior of the compounds of interest. The success of the models used to correlate experimental data is largely limited by the reliability of the data. However, there are wide variations in both the levels and trends of solubility data of even the most

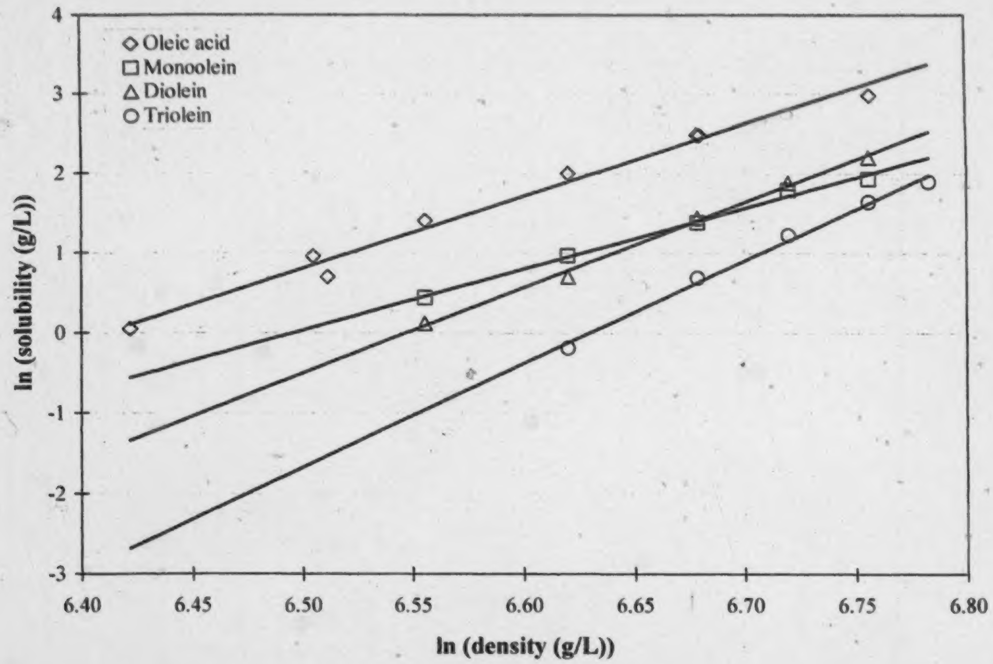

Figure 2.4. Solubility isotherms for olein glycerides in $SCCO_2$ at 323 K (Solid lines represent regression results obtained using equation 2.2).

studied compounds, which limits the applicability of the existing database. These discrepancies are mainly due to differences in the purity of samples and limitations of the experimental techniques used. Extreme caution should be exercised while measuring lipid solubilities to eliminate the potential sources of error. Degradation of the samples during sample handling, processing and storage should be avoided. Isomeric purity of the solutes should also be considered as different physical and chemical properties of the isomers will be reflected in their solubilities.

The problem is further complicated by the lack of phase behavior information, such as phase transitions, which is essential for the accurate interpretation of the data. Although the physical state of the solute seems to be the main factor affecting the solubility behavior, information on the melting behavior of pure lipids in $SCCO_2$ media is lacking in the literature.

Most of the studies on binary mixtures of lipid classes in $SCCO_2$ only involve the sampling of the supercritical phase. The composition of the liquid phase is also necessary in order to determine the complete phase behavior. MG and DG are the least studied classes of lipids, although information on their phase behavior is essential for the design of processes such as fractionation of glyceride mixtures or refining of vegetable oils, which are of great commercial importance. Although mixed TG are the main components in many fats and oils products, there are no studies on the binary solubilities of such compounds in $SCCO_2$, which is mainly because of the high cost of pure samples.

The knowledge of the effect of operating conditions and solute properties on the solubility behavior is essential for the design of fractionation processes. The existence of a crossover region in multicomponent systems between the crossover pressures of two compounds, where an isobaric change in temperature affects the solubilities of the two compounds in opposite directions, can be used to achieve separation of a mixture into its components [60] and merits further consideration.

The effect of solute properties on the solubility is largely dependent on the operating conditions. In an analogous glyceride series, the net effect of the factors, vapor pressure and polarity on solubility is dependent on the density of the solvent. At low densities, the effect of the vapor pressure dominates, whereas at higher densities, the polarity effect becomes more dominant. The crossover behavior is significant in terms of the fractionation of these compounds.

The parameters of the Chrastil equation estimated in this study can be used to obtain preliminary information on the solubility characteristics of pure lipids. However, it should be noted that these results can only be used as guidelines for real systems because various factors such as solute entrainment and component interactions will affect the phase behavior of multicomponent mixtures. Future emphasis, therefore, should be on the solubility behavior of multicomponent mixtures to enhance our understanding of the behavior of fats and oils products in $SCCO_2$.

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3. CORRELATING THE SOLUBILITY BEHAVIOR OF MINOR LIPID COMPONENTS IN SUPERCRITICAL CARBON DIOXIDE[1]

3.1. INTRODUCTION

Successful application of supercritical fluid (SCF) technology to fats and oil processing requires a good understanding of solubility behavior of and physical properties of lipid components. The study of simpler binary systems can provide valuable information on the fundamentals of solubility behavior, which can further be used in the interpretation of multicomponent behavior. Thus, general trends of solubility behavior of fats and oils components as affected by operating conditions and solute properties can be determined by a systematic study of binary phase behavior.

Fats and oils are complex mixtures of fatty acids (FA), fatty acid esters (FAE), mono, di- and triglycerides (MG, DG, TG) and minor components such as vitamins, sterols, phospholipids, pigments and hydrocarbons. The minor lipid components make up the unsaponifiable fraction, which is determined by methods based on extraction from the residue left after the saponification of fats and oils [1]. Table 3.1 shows the unsaponifiable content of selected fats and oils.

There has been growing interest on minor bioactive lipid components due to the mounting evidence on their health benefits [4, 5]. Increasing consumer demand for "natural" products [6] has put the focus on alternative processing technologies such as $SCCO_2$ processing. Solubility behavior of binary systems of pure lipids belonging to the major lipid classes and $SCCO_2$ has been studied previously (Chapter 2). However, a

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Table 3.1. Unsaponifiable, total tocopherol and total sterol content of selected vegetable oils [2, 3].

	Soybean	Canola	Palm	Rice bran	Wheat germ
Unsaponifiable (%)	0-1.5	0-2	0-1.2	3-5	2-5
Tocopherols (mg/kg)	573-3363	424-2680	4-1079	400	1350-2500
Tocotrienols (mg/kg)	0-172	-	98-1500	700	20-400
Total sterols (mg/kg)	1840-4090	4820-11280	362-627	10550	5500

systematic study on the solubility behavior of minor lipid components in $SCCO_2$ has not been reported. Therefore, the objectives of this study were: a) to review the properties and processing of minor lipid components, b) to correlate the solubility data of minor lipid components using Chrastil's equation to establish general trends of solubility behavior as affected by operating conditions and solute properties, c) to compare the solubility behavior of fats and oils components, and d) to assess implications of the findings for extraction and fractionation processes to isolate minor lipid components. The minor lipid components studied included β-carotene, tocopherols, phytosterols (plant sterols) and squalene. Phospholipids and chlorophylls were not included as they are sparingly soluble/practically insoluble in pure CO_2 at practical operating conditions [7, 8].

3.1.1. Solute properties

The study of the solubility behavior of lipid components in $SCCO_2$ requires knowledge of physical properties such as molecular weight and vapor pressure (boiling point) as summarized in Table 1.3 for minor components of interest.

β-Carotene (Fig. 1.4) belongs to the family of carotenoids, which are widely distributed pigments responsible for the red, yellow and orange colors of fruits, roots, flowers, fish, invertebrates, birds, algae, bacteria, molds and yeast. Carotenoids include two classes of compounds: C40 polyunsaturated hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls). The presence of conjugated double bonds in their structure accounts for their pigmentation, UV-visible absorption, antioxidant activity and instability (susceptibility to oxidation and geometric isomerization). Heat, light, acids, refluxing in an organic solvent, melting of the crystals and treatment with iodine promote *cis-trans* isomerization [9, 10]. Isomerization of *trans* carotenoids, which is the usual configuration in nature, to *cis* forms increases the solubility and decreases color intensity, melting point and provitamin A activity [9, 10]. Oxidation, the major degradation mechanism of carotenoids, is accelerated by light, heat, the presence of unsaturated fats, peroxides, metals such as iron, copper and manganese, and exposure to organic solvents and enzymes [11, 12]. Degradation of carotenoids, therefore, can be limited by exclusion of light and oxygen, addition of antioxidants, using freshly purified solvents with minimum heating and refrigeration [12, 13]. Major functions of carotenoids in nature include being accessory pigments in photosynthesis and photoprotection [12, 13]. The major role of carotenoids in human health is as vitamin A precursors, with β-carotene having the highest vitamin A activity [14]. In addition to being the most potent biological quencher of singlet oxygen, β-carotene also acts as a chain breaking antioxidant [15-17]. At high oxygen partial pressures however β-carotene has prooxidant properties [17].

The Vitamin E family of compounds (Fig. 1.5) consists of four tocopherol (α, β, δ, γ) and four tocotrienol (α, β, δ, γ) isomers. Tocopherols consist of a chroman ring and a long saturated phytyl chain. Tocotrienols have identical chroman rings to the corresponding tocopherols, but unsaturated side chains. The four isomers (α, β, δ, γ) differ only in the number and position of the methyl groups. The richest natural sources of tocopherols are lipid-rich plant products, including cereals (bran/germ portion of grains), oilseeds, nuts and vegetables, such as peas, beans and carrots [18]. Tocopherol content of selected vegetable oils is given in Table 3.1. Tocopherols are viscous, pale yellow oils at room temperature. Under inert conditions (in the absence of oxygen) they are stable to heat, acid and alkali, whereas they are slowly oxidized by atmospheric oxygen. Oxidation is accelerated by iron salts [19]. Vitamin E, a major lipid-soluble antioxidant, functions as a chain breaking antioxidant due to its scavenging of free radicals by the OH group on the chroman ring [20]. A major antioxidant function of Vitamin E in the human body is the protection of unsaturated FA in cell membranes that are important for membrane function and structure by inhibiting lipid peroxidation [21].

Vegetable oils (Table 3.1) and products derived from oils are the richest natural sources of sterols, followed by cereal grains, cereal-based products and nuts [22]. Phytosterols comprise a major portion of the unsaponifiable matter of most vegetable oils. They are crystalline, neutral alcohols of high melting points. The major plant sterols, β-sitosterol, campesterol, stigmasterol and brassicasterol differ from cholesterol in that they are substituted at position 24 by either a methyl or an ethyl group; furthermore stigmasterol and brassicasterol contain a double bond between carbons 22 and 23 (Fig. 1.6). Chemical and physical properties of sterols have been reviewed by Piironen et al. [22]. Phytostanols are saturated derivatives of phytosterols and occur in significant amounts in corn bran and corn fibre oils [23]. In oils, phytosterols occur as free sterols, sterol esters (esterified to a FA or a phenolic acid) and glycosides (linked to a carbohydrate). Corn bran, corn fibre and rice bran oils are rich sources of ferulate esters [3, 23, 24]. Oryzanol, a mixture of ferulic acid sterol esters, is the main sterol in rice bran oil. Functions of sterols in plants include regulation of the fluidity of membranes and adaptation of membranes to temperature, cellular differentiation and proliferation [22]. Sterols show antioxidant activity in oils at frying temperatures [25].

Hydrocarbons are present in trace amounts in all edible oils. Squalene, a highly unsaturated linear triterpene (Fig. 1.7), is the main hydrocarbon of olive, rice and fish oils (shark liver oil) [26]. It is an important intermediate in the biosynthesis of cholesterol. Its thermal and oxidative instability leads to darkening, odor formation and increase in viscosity due to polymerization [26, 27]. Squalene has been shown to have antioxidant activity and thus prevent lipid oxidation [28].

3.1.2. Health benefits

Minor lipid components are receiving increased attention due to their health benefits. There has been growing evidence on the protective action of antioxidants, vitamin E and β-carotene, against free radical mediated cell damage, which has been implicated in the development of various degenerative diseases and conditions such as cancer, cardiovascular disease, aging, cataracts, age related macular degeneration, and against lipid peroxidation associated with strenuous exercise and air pollution [14, 29-32].

Although α-tocopherol has been the focus of most research in the past, other tocopherols and tocotrienols are also being investigated [33, 34]. Tocotrienols have been reported to have more antioxidant activity than tocopherols in biological systems [21] in addition to cholesterol lowering, anti-carcinogenic and neuroprotective properties, which may not be related to their antioxidant function [34].

Although the cholesterol-lowering ability of phytosterols has been known since 1950s [35], their use was limited by the poor solubility of free sterols and their crystalline nature [36]. The production of lipid-soluble fatty acyl derivatives through esterification enabled the incorporation of phytosterols into functional foods, such as margarines and spreads [37, 38] and renewed the interest in their health benefits, which is reflected in the increased level of research activity on this subject [39-42]. Although the focus was initially on the phytostanols, recent research indicating equivalent efficacy of sterols [43] led to simplification of the manufacturing process due to elimination of the hydrogenation step. The recent issuance of a health claim for phytosterols and phytostanols by the U.S. Food and Drug Administration [42], indicating that they can reduce the risk of heart disease has increased the interest in phytosterols as functional food ingredients.

Squalene has been reported to have anti-carcinogenic activity [44, 45]. The minor lipid components also contribute to health benefits associated with oils. High squalene content of olive oil has been proposed to be a major factor in its effect on reducing cancer risk [45]. The cholesterol lowering properties of rice bran oil [46] and corn fiber oil [47] were partly attributed to their minor components, sterols and tocotrienols for rice bran and sterols for corn fiber oils.

3.1.3. Commercial uses and processing

3.1.3.1. Conventional processing and uses

β-Carotene is the most commercially significant of the carotenoids and is isolated from natural sources (usually algae and carrots) or synthesized [48]. The major commercial uses of β-carotene are as food colorant and vitamin supplement as well as pharmaceutical, cosmetic and therapeutic uses [49]. The stability problems and the difficulties associated with handling of the pure crystalline carotenoids led to the development of a wide range of stable and easy-to-use products such as suspensions of micronized crystals in highly purified vegetable oil, emulsions and dry gelatin protected beadlets [50].

Carotenoids can be extracted from the dried tissue with water-immiscible solvents, such as diethyl ether and light petroleum; however, aqueous solvents such as ethanol and acetone are used for extraction from fresh tissue where they act both as a dehydrating and extraction solvent [12]. Recovery of carotenoids from crude palm oil, one of the richest sources of carotenoids, directly and through esterification has been investigated using a number of methods such as saponification, the iodide method, vacuum distillation, molecular distillation, solvent extraction, solvent crystallization and fractionation, adsorption and gel permeation [49]. The difficulty, inefficiency and high cost of most of these methods were attributed to the sensitivity of carotene to oxygen, light, heat and acid degradation, low concentration in natural sources, low selectivity of physical separation methods due to similar physical properties (solubility, polarity, molecular weight) of carotenes and TG and irreversible changes induced by chemical methods [49].

Synthetic Vitamin E (all-rac α-tocopherol), which is an equimolar mixture of all eight stereoisomers of α-tocopherol accounts approximately for 80% of the worldwide market, which is estimated at 25,000 metric tons. The large-scale industrial synthesis is relatively cheap and simple and consists of three major steps: the preparation of the aromatic building block, the production of the side chain component and the condensation reaction. The feed industry is the main sector of applications (71%) followed by pharmaceutical (24%), food (2%) and cosmetic industries (3%) [51].

Tocopherol esters (acetate, succinate), which are more stable to oxidizing agents such as air, light and metals than free tocopherols, are used commercially to fortify foods or as nutritional supplements. However, tocopherols can be used as food antioxidants only in their free, unesterified form as the active site is the 6-hydroxyl group on their chroman ring [52].

Natural vitamin E, which is a concentrate of mixed tocopherols, is derived primarily from deodorizer distillates and used almost exclusively for human consumption, with only about 1% of the market going to animal feed and a small portion to cosmetics [51]. Concentrates of tocopherol mixtures are used as antioxidants or marketed as vitamin E after chemical methylation (to convert β-, δ-, γ- to biologically more active α-tocopherol) and subsequent acetylation to obtain the relatively stable D-α-tocopheryl acetate [18].

Phytosterols have been traditionally used in the pharmaceutical and cosmetic industries as starting materials or as intermediates for the synthesis of drugs and hormones [53]. They are increasingly being used as cholesterol lowering ingredients in functional foods, pharmaceuticals and nutritional supplements. Their emergence as functional food ingredients led to the development of new methods for their introduction into aqueous systems, expanding their application areas [54]. Production of edible oils rich in phytosterols with cholesterol lowering properties such as corn fiber oil [55] and designer oils [56] are also being commercialized.

The major commercial source of tocopherols and phytosterols is deodorizer distillate, a by-product of the deodorization step in vegetable oil refining. It is a complex mixture consisting of sterols, tocopherols, free fatty acids (FFA), glycerides, aldehydes, ketones and other volatiles [53, 57]. The recovery of sterols and tocopherols from such a complex mixture requires a series of physical and chemical treatments such as urea adduct formation, liquid-liquid extraction, distillation and alkali saponification.

Separation of FFA from the deodorizer distillate can be facilitated by their conversion to esters of lower alcohols, such as methanol in the presence of an acid catalyst such as HCl. Prior to the esterification step, the distillate can be saponified and acidified to obtain a mixture of FFA, tocopherols, sterols [58] or alternatively a concurrent splitting and esterification step can be used [59]. Using large quantities of alkali to hydrolyze and saponify the distillate reduces the tocopherol yield considerably as tocopherols are labile in the presence of alkali [60]. When the esterification reaction is acid-catalyzed, subsequent alkali neutralization and treatment to remove the salt are required. Enzyme catalyzed esterification has been investigated as an alternative [61-63]. The esterification step can be preceded with an enzyme-catalyzed hydrolysis step [63]. An alternative approach uses an esterification step and a transesterification step [64, 65]. After esterification, the volatile fraction can be removed from the tocopherols and sterols using distillation methods such as vacuum [59, 62] or fractional distillation [63].

Molecular distillation without any pretreatment step was also investigated for the isolation and purification of sterols and tocopherols and found to be more efficient than solvent extraction and sterol crystallization [66]. Due to the low temperatures and high vacuum conditions used in molecular distillation the tocopherols are protected from degradation; however, special equipment is required to achieve these conditions. FFA can also be removed from the tocopherols by converting them into alkali metal salts in an organic solvent such as acetone or ethyl acetate, which does not dissolve them [67].

Sterols can be separated from the tocopherols by crystallization, solvent extraction or adduct formation with crystallization [68]. Brown [60] used a two-step fractional liquid-liquid extraction using polar and non-polar solvent pairs to separate tocopherols and sterols in the deodorizer distillate. Esterification of sterols with FFA in deodorizer distillates can also be used to facilitate their separation from tocopherols [69-71].

FAE of sterols/stanols are prepared via chemical or lipase catalyzed esterification of free sterols with FA [72-74] and transesterification with fatty acid methyl esters or TG [75]. Alternatively, sterols in the deodorizer distillates can be converted to sterol esters via a number of steps including hydrolysis, esterification or transesterification [76]. The sterol esters can be further purified using deacidification, flash chromatography and solvent fractionation [76].

Squalene has been a popular item in health food stores sold in capsule form with claims to enhance oxygenation of the blood, facilitate detoxification, strengthen immune system, protect against cancer and provide increased longevity. It is also used widely in several cosmetic applications as a carrier for lipid-soluble components since it is absorbed by the skin easily [27]. Other uses include as a bacteriocide, as an intermediate in the manufacture of pharmaceuticals, organic coloring matter, rubber chemicals, aromatics and surface-active agents, in finishing natural and artificial silk, as a lubricant and in filling certain types of thermometers [26]. Squalene has been traditionally extracted from shark liver oil [77]. The uncertainty of its continued availability due to international concern for the protection of marine animals led to the investigation of other potential sources such as amaranthus grain [78, 79]. Short path distillation [78] and isolation of unsaponifiables followed by column chromatography [79] have been studied for the enrichment of squalene in amaranth seed oil.

Minor lipid components are removed from the oils during traditional oil refining procedures. The effect of conventional refining on minor lipid components has been studied by various researchers [80-84]. Increased recognition of their bioactivity has led to the reevaluation of conventional processes and the development of new processes to maximize the retention of these bioactive components [85-87] and resulted in increased utilization of by-products as raw materials for the concentration of these high-value components [88].

3.1.3.2. Processing with $SCCO_2$

Growing evidence on the health benefits of the minor lipid components and increasing consumer demand for "natural" ingredients have put the focus on the extraction/concentration of these components from natural sources using environmentally friendly technologies. SCF technology has been an attractive alternative to traditional methods for the processing of minor lipid components as it is an environmentally friendly process offering various advantages, such as operation at low temperatures, absence of oxygen and lack of solvent residues in the final product. The use of $SCCO_2$ extraction and fractionation processes for the concentration/enrichment of minor lipid components from oils and oil by-products and for the refining of oils has been discussed in Chapter 1 (Section 1.2.2.3.3).

$SCCO_2$ extraction of minor lipid components from vegetables has also been investigated. $SCCO_2$ has been used to extract β-carotene from a variety of sources such as sweet potatoes [89], non-edible leaves [90], leaf protein concentrates [91], carrots [92-96] and *Dunaliella* algae [97, 98]. Recovery of carotenoids from marjoram [106], tomatoes [107] and tomato processing by-products [101, 102] by $SCCO_2$ extraction has also been investigated. $SCCO_2$ extraction of stigmasterol and squalene from medicinal plants [103] and FA and sterols from plant tissues and sediments [104] were reported.

Supercritical extraction/fractionation protocols used for analytical purposes should not be overlooked since some approaches may have implications for process development. At the analytical scale, SFE and supercritical fluid chromatography (SFC) using $SCCO_2$ have also been used extensively for the determination of minor lipid components [105-108]. Snyder et al. [106] developed an analytical method using a multistep SFE procedure and SFC for sterol analysis in various lipid-laden matrices such as seed oils, margarine, corn germ oil and corn fiber oil. Marsili and Callahan [108] obtained equal or greater recoveries of carotene from vegetables using $SCCO_2$ than those obtained with traditional solvent extraction methods.

$SCCO_2$ has also been used as a reaction medium in the production of minor lipid components such as sterol esters [109]. Other applications of $SCCO_2$ processing include coupling of SFE with nanofiltration for the purification of β-carotene from carrot oil or carrot seeds [110], gas antisolvent process to separate and purify β-carotene [111], the RESS process (rapid expansion of supercritical solution) to obtain a homogeneous mixture from a test mixture of caffeine and carotene (powder coprecipitation) [112] and encapsulation of β-carotene in microspheres via $SCCO_2$ [113].

3.2. SOLUBILITY DATA AND CORRELATION

Literature solubility data of binary systems of minor lipid components (β-carotene, tocopherols, stigmasterol and squalene) and $SCCO_2$ were compiled (Table 3.2) and correlated using Chrastil's model [129] as given in eq 2.1 and 2.2 and discussed in detail in Section 2.2.3. Chrastil's model was adopted for the systematic study of binary solubility behavior of lipid components as it is a simple empirical model that does not require information on the properties of lipid components. Its value however is limited for predictive modeling of solubility data as discussed in Chapter 2.

In this study, model parameters a, b and k -valid for the whole temperature range- (using eq 2.1) and parameters k' and b' at each temperature (using eq 2.2) were estimated using a multivariate regression analysis of the SAS statistical software package [140]. Data for solutes with unknown purities or with purities <95% were excluded from the analysis to improve the goodness of fit of the model. Data, which consistently deviated from the rest of the database [120, 127] were also excluded. The range of experimental conditions in which solubility increased with pressure was used for the correlation. CO_2 density data used in the model as well as for the conversion of solubility data to the required units (g/L) were obtained using the density calculator in the Supercritical Fluid Solubility Calculator Software, Version 1.01 [141]. These density values were

Table 3.2. Literature solubility data for binary systems of pure minor lipid components and $SCCO_2$.

Solute	No. of data[a]		Data presented as[b]	T (K)	P (MPa)	Purity[c] (%)	Experimental method[d]	Ref
	liquid	vapor						
Pigment								
β-carotene	-	g	%w/w	288-328	5-80	97	St	114
	-	g	mol/v	288.7-309	10-160	97	St	115
	-	-	-	308-350	8-180	97.1	St	116
	-	35	mol/v	307.6, 322.9	8-180	>97	St	117
	-	9	mol/v	313, 343	6.2-34.3	n.r.	St	118
	-	23	w/w, m.f.	313-353	20-35	95	St	119
	-	24	w / v	298, 313	9.8-30.9	>95	St	120
	-	13	m.f.	313-333	12-28	97	D	121
	-	21	m.f.	313-333	12-29.9	95	D	122
	-	3	w/w	313-333	30	>98	D	123
	-	26	w/v, m.f.	308-323	997-29.8	96	D	124
	-	g	m.f.	323	15-22.3	99.5	D	125
	-	27	m.f.	310-340	9.7-26	80-98	R	126
	-	16	m.f.	313-343	21.2-43.9	n.r.	R	127
Tocopherols								
α-tocopherol	-	24	w/w, m.f.	313-353	19.9-34.9	>98	St	119
	-	17	m.f. + w/v	298, 313	10.0-18.3	>98	St	128
	-	15	w/v	313-353	10.1-25.3	n.r.	St	129
	g	g	m.f.	313-353	8-30	97	St	130
	-	g	wt %	313-333	10-20	n.r.	D	131
	20	20	mole %	313, 333	9.9-24.2	95	R	132
	48	50	m.f. + w/v	298-333	9.1-26.8	98	R	133
	22	32	mole %	303-353	9.5-35.2	98	R	134
δ-tocopherol	-	24	w/w, m.f.	313-353	19.9-34.9	90	St	119
Sterols								
stigmasterol	-	19	m.f.	308-333	9.1-30.4	95	D	135
β-sitosterol	-	g	w/v	293-353	10-90	n.r.	n.r.	136
Hydrocarbon								
squalene	8	19	m.f.	313-333	10-25	n.r.	D	137
	8	9	w/w^V, m.f.^{L}	323, 333	10-30	98	D	138

[a] No of data: g., data presented only in graphs

[b] Data expressed as: m.f. mole fraction

[c] Purity: n.r., not reported

[d] Experimental method: St, static; D, dynamic; R, recirculation

comparable to the density values obtained using Dionex SFC/GC Control Software in Chapter 2.

3. 3. RESULTS AND DISCUSSION

In Chapter 2, literature solubility data for major lipid components were correlated using Chrastil's equation and analyzed using solubility versus pressure/temperature graphs to determine the effect of operating conditions and solute properties on solubility behavior. In this chapter, a similar approach is extended to minor lipid components.

3.3.1. Solubility data and correlation

Model parameters estimated using eq 2.1 and 2.2 are presented in Tables 3.3 and 3.4, respectively. Values of parameter 'k' were in the range of 4.9-10.6 for β-carotene, 4.5-9.6 for α-tocopherol, 4.9-8.0 for stigmasterol and 7.3-7.6 for squalene in the temperature ranges investigated. Poor correlation, reflected in low R^2 values was partly due to discrepancies between literature data. Variation between literature solubility data has been observed for various lipid components and has been attributed to the purity of the samples and the limitations of the experimental techniques used as discussed in Section 2.2.1. Solute loss may occur during depressurization due to precipitation of the solute inside valves and tubing. The precipitated solute can be recovered by washing with a solvent but this step is not done in all studies. Another possible source of error is the sampling/quantification step. Off-line sampling involves collection of the solute in a cooled collection flask such as a U-tube, in a solvent or sorbent trap followed by gravimetric, chromatographic or spectrophotometric analysis. With on-line sampling, solubility is measured prior to the depressurization step using a chromatography (SFC, HPLC) or spectrophotometric system coupled to the extractor, eliminating solute loss and additional sample handling. One of the possible drawbacks of the on-line systems is the potential saturation of the UV detectors that can occur at high concentrations [122].

Table 3.3. Model parameters for pure minor lipid components estimated using equation 2.1 (ln c = k ln d + a/T + b).

Solute	k ±standard error	a±standard error	b±standard error	R^2
β-carotene	7.2±0.3	-8844.3±411.6	-26.8±1.8	0.899
α-tocopherol	5.7±0.3	-3687.4±381.6	-24.2±1.6	0.810
stigmasterol	5.8±0.4	-7300.5±718.9	-18.1±2.6	0.926
squalene	7.3±0.1	-4131.0±338.6	-33.0±1.3	1.000

Table 3. 4. Model parameters for pure lipid components estimated using equation 2.2 (ln c = k′ ln d + b′).

Solute	T (K)	k′ ±standard error	b′±standard error	R^2	Data from ref
Pigment					
β-carotene	308	4.9±0.4	-39.8±2.6	0.9239	117, 124
β-carotene	313	8.6±0.5	-64.3±3.4	0.9144	119, 121-124
β-carotene	318	9.9±0.1	-72.3±0.9	0.9993	124
β-carotene	323	6.8±0.3	-51.9±2.0	0.9407	117, 121-124
β-carotene	333	10.6±0.2	-76.1±1.3	0.9935	119, 121-123
β-carotene	353	9.2±0.7	-64.9±4.6	0.9719	119
Tocopherol					
α-tocopherol	306	7.0±0.7	-45.4±4.9	0.9320	123
α-tocopherol	313	8.9±0.6	-58.0±3.7	0.8508	119, 128, 132-134
α-tocopherol	323	4.5±0.5	-27.8±3.0	0.8686	133, 134
α-tocopherol	333	5.0±0.4	-31.3±2.6	0.8600	119, 132, 133
α-tocopherol	343	9.6±1.9	-61.3±12.9	0.9254	134
α-tocopherol	353	5.6±0.4	-34.1±2.6	0.9460	119, 134
Sterol					
stigmasterol	308	6.8±0.3	-48.2±2.3	0.9879	135
stigmasterol	323	8.0±0.4	-55.5±2.4	0.9922	135
stigmasterol	333	4.9±0.4	-33.9±2.7	0.9712	135
Hydrocarbon					
squalene	323	7.3±0.1	-45.8±0.6	0.9995	138
squalene	333	7.6±0.4	-47.2±2.9	0.9934	138

Alternatively, quantification can be carried out using physicochemical methods, such as spectroscopic methods, inside the equilibrium cell under pressure [115, 116]. These factors all contribute to the observed discrepancies, especially at low solubility levels, such as those observed for β-carotene (Fig. 3.1).

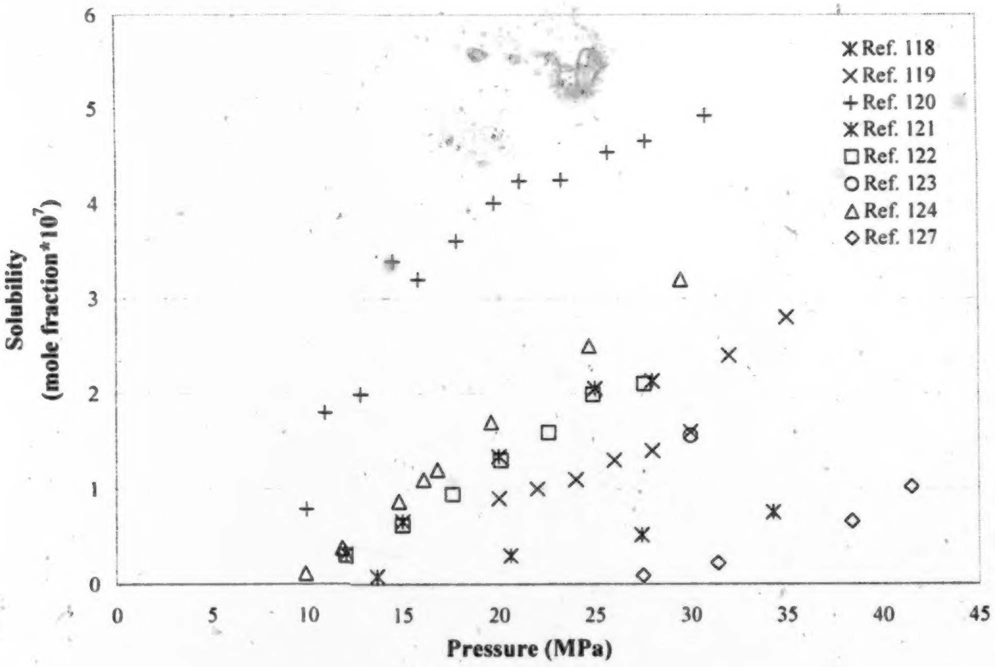

Figure 3.1. Solubility isotherms of β-carotene at 313 K.

Knowledge of the physical properties of the solutes of interest (Section 3.1.1) is required to minimize any possible sources of variation. The effect of solute purity on solubility in $SCCO_2$ has been well documented as discussed in Chapter 2; therefore, sample purity should be as high as possible and should be reported. Impurities in the samples may also arise from degradation of the samples due to improper handling. Degradation through oxidation (for β-carotene, tocopherols and squalene) or isomerization (for β-carotene) can occur during the extraction, depressurization or recovery steps. Chang and Randolph [142] reported that it was not possible to carry out measurements in CO_2 as β-carotene was oxidized by CO_2 producing β-carotene related epoxide compounds. However, their findings were not confirmed by Jay et al. [114], since they did not find any evidence of β-carotene oxidation by CO_2 at high pressure and temperature. Cocero et al. [123] later pointed out that this reaction was not possible from a chemical view point. Although the exclusion of oxygen during $SCCO_2$ extraction has been recognized as one of the advantages of SFE, oxidative degradation of β-carotene during SFE of alumina beads impregnated with β-carotene has been reported [123]. The authors noted that even the highest purity CO_2 would contain enough oxygen to cause oxidation. Manninen et al. [143] observed tocopherol loss during the SFE of cloudberry seed oil and noted the prooxidant activity of trace elements in the plants as a possible cause. β-Carotene can also undergo isomerization during SFE [89] and solubility measurements [115], resulting in the formation of *cis* isomers. Solvent traps used to collect the solute, such as hexane [122] have also been reported to cause isomerization or degradation of the sample. High degree of dispersion of β-carotene after the expansion of the supercritical solution has been reported to promote chemical attack [122].

Isomerization affects the solubility measurements as *cis* isomers of β-carotene are more soluble than *trans* isomers in $SCCO_2$ [125]. It should be noted that solubility measurements of *cis* isomers were carried out using algae extracts containing a mixture of *cis* and *trans* isomers of β-carotene.

Exposure of the samples containing minor lipid components to oxygen, heat and light should be minimized during processing, analysis and storage to limit degradation. Highest purity CO_2 should be used. Using dark colored vials or covering sample tubes/vials with aluminum foil and purging the headspace with nitrogen are recommended. The collection flask should be kept at a low temperature. Addition of antioxidants such as butylated hydroxytoluene (BHT) can minimize oxidative degradation [123, 125]. However, antioxidants should not be added before the solubility measurements as they will interfere with the measurements. Experimental precautions that can be used to minimize the effect of sample degradation on solubility measurements include using sudden releases of the gaseous phase to blow off any impurities from the cell before adjusting the temperature and pressure [115] and using a fresh batch of sample for each single data point to avoid accumulation of carotene isomers [116].

Physical state of the solute was determined to have a major influence on the solubility behavior of major lipid components as discussed in Chapter 2. Melting point of a solid solute decreases when it is compressed in a supercritical fluid. Therefore, the melting point of the solute and possible melting point depression should be considered while studying the solubility behavior. The melting points of the studied solutes are listed in Table 1.3. While squalene and α-tocopherol are liquid, stigmasterol and β-carotene are solid under the studied experimental conditions. Melting of stigmasterol and carotene is not expected in binary mixtures of $SCCO_2$ under practical operating conditions due to their high melting points (170 °C and 183 °C, respectively).

The crystalline nature of β-carotene can also affect its solubility. Sakaki [124] noted that amorphous β-carotene obtained by evaporation from a solution in n-hexane together with alumina beads was more soluble than crystalline β-carotene as evidenced by its lower enthalpy of fusion.

3.3.2. Solubility behavior

3.3.2.1. Operating conditions

3.3.2.1.1. Effect of pressure

Solubility of all solutes studied increased with pressure; however, the pressure dependence of solubility varied between solutes and with temperature. Reaching a solubility maximum with pressure has been reported for amorphous β-carotene in the temperature range 288.7-309 K at a pressure of about 100 MPa [115], for crystalline β-carotene at approximately 60 MPa and 307.6 K [116] and for β-sitosterol in the density range of 1040-1080 g/L [136]. Solubility maxima have also been reported for stearic acid around 30 MPa at 313 K [144] and at pressures of 28-30 MPa at 318 K [145] and for vegetable oils at around 100 MPa at 313-333 K [146].

3.3.2.1.2. Effect of temperature

The effect of temperature on solubility at constant pressure is due to two mechanisms: the increase in vapor pressure with temperature, resulting in a solubility increase and the decrease in solvent density, causing a decrease in solubility. These opposing factors result in the crossover of solubility isotherms. The dominant mechanism is dictated by pressure such that solubility decreases with temperature (also referred to as retrograde behavior) at pressures lower than the crossover value and increases with temperature at higher pressures.

Similar to lauric acid and FAE (Section 2.3.2), squalene (Fig. 3.2) showed retrograde solubility behavior over the entire experimental range indicating crossover pressures higher than the maximum pressures used. In general, solubility of α-tocopherol decreased with temperature in the experimental conditions tested. There appears to be a crossover of isotherms at higher pressures used. For example, tocopherol solubility data of Johannsen and Brunner [119] show a crossover between 313 K and 333 K isotherms at around 30 MPa and between 333 K and 353 K isotherms at around 35 MPa (Fig. 3.3). Similar to solid FA (Section 2.3.2), solubility of stigmasterol (Fig. 3.4), β-carotene and β-sitosterol, which were solid under the experimental conditions, increased with temperature indicating that the crossover pressures were lower than or close to the minimum pressure used. Mendes et al. [122] noted a crossover pressure of 15-17.5 MPa in the temperature range of 313-333 K for synthetic (*trans*) β-carotene. Crossover pressure of natural carotene from *Dunalliella salina*, which is a mixture of *cis* and *trans* isomers (ratio of 1.75) was around 30 MPa [98], demonstrating an effect of mixture composition on crossover pressure.

Johannsen and Brunner [119] discussed the effect of temperature on solubility of δ-tocopherol and β-carotene at constant pressure. At pressures >20 MPa, δ-tocopherol solubility increased with temperature in the range of 313 to 333 K, whereas at temperatures >333 K solubility decreased with temperature at constant pressure. At 20 MPa, solubility decreased with temperature over the entire temperature range. A solubility maximum, though not as evident, was also observed for α-tocopherol at 333 K

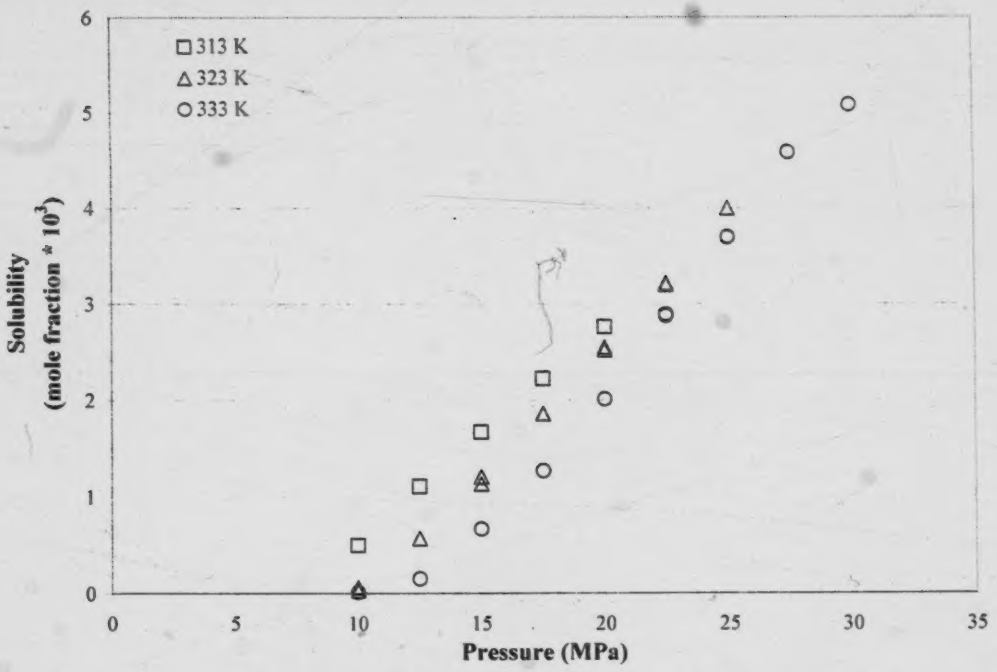

Figure 3.2. Solubility isotherms of squalene (Data from Refs. 137 and 138).

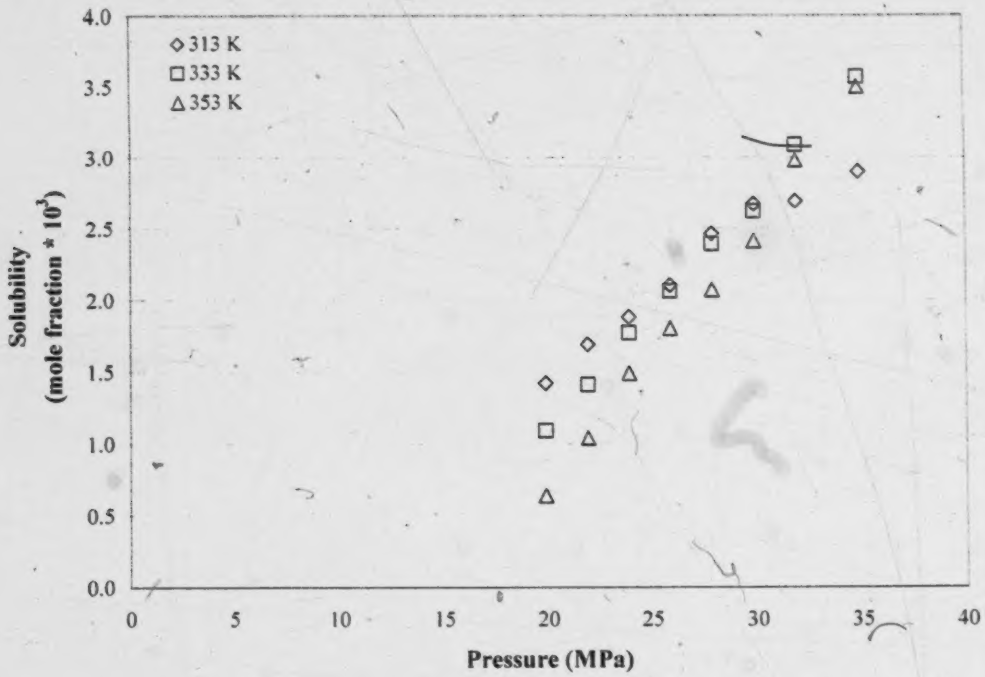

Figure 3.3. Solubility isotherms of α-tocopherol (Data from Ref. 119).

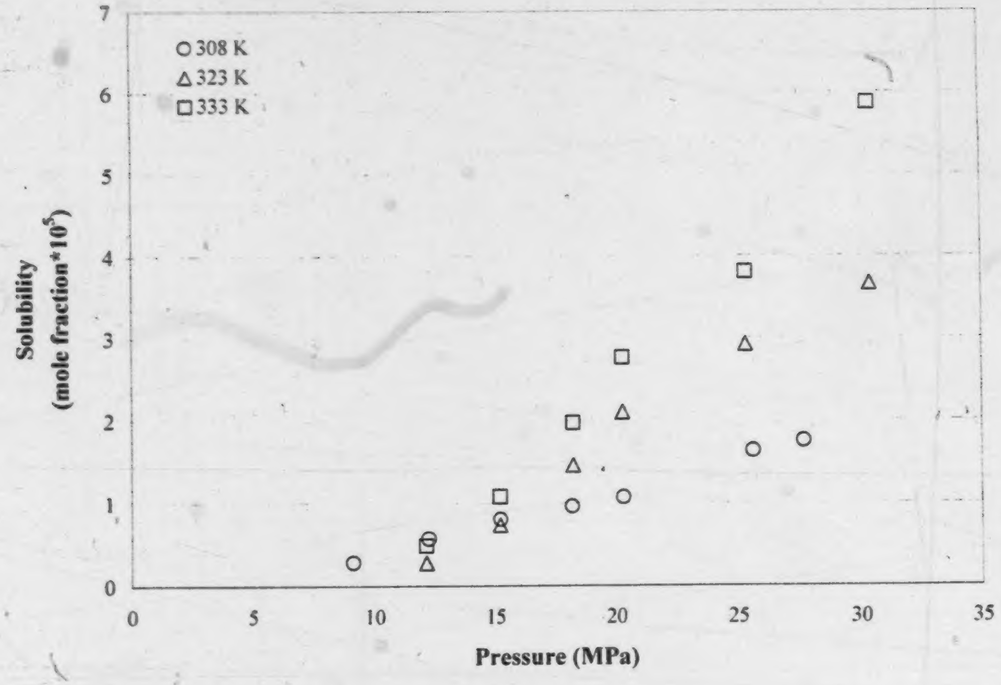

Figure 3.4. Solubility isotherms of stigmasterol (Data from Ref. 135).

at pressures >30 MPa (Fig. 3.5). Solubility maximum with temperature leads to a crossover pressure range such as that observed for α-tocopherol (Fig. 3.3). The solubility of β-carotene increased with temperature at all pressures (313-353 K, 20-32 MPa). Solubility maxima of isobars close to the crossover point has been observed for several lipids. Tilly et al. [147] reported a maximum extraction rate for a vegetable oil mixture at 333 K, which was more pronounced at 27 MPa than at 21 MPa. Solubility maxima have also been reported for triolein 318 K in the pressure range of 8-21 MPa [148] and for trilaurin at 313 K in the pressure range of 25-30 MPa [149].

3.3.2.2. Solute properties

Estimated model parameters were used to compare the solubility behavior of minor lipids with components of olein glyceride series (oleic acid and triolein) as representatives of the major lipid classes found in fats and oils (Fig. 3.6). The comparison of solubility behavior of components of olein glyceride series (oleic acid, mono, di- and triolein) was reported in Chapter 2. In the olein glyceride series, oleic acid was the most soluble solute followed by mono- and diolein, for which relative solubilities were density dependent. Triolein was the least soluble solute in this series. Solubility behavior in $SCCO_2$ is determined by vapor pressure and intermolecular interactions. In a homologous series where the intermolecular interactions are similar (such as FA), molecular weight is a good indicator of vapor pressure/solubility such that vapor pressure and hence solubility decreases with increasing molecular weight. Wong and Johnston [135] measured the vapor pressures of cholesterol and stigmasterol and observed that at 308 K the ratio of vapor pressures was similar to the solubility ratio in $SCCO_2$. They also observed similar enhancement factors (ratio of actual solubility to that predicted by ideal gas law) for

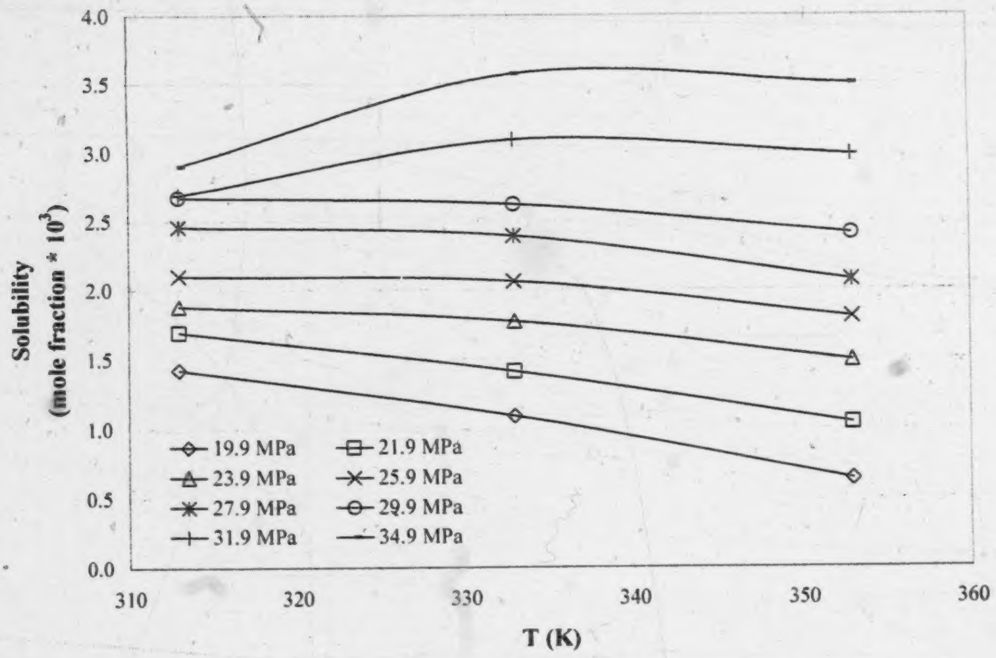

Figure 3.5. Solubility isobars of α-tocopherol (Data from Ref. 119).

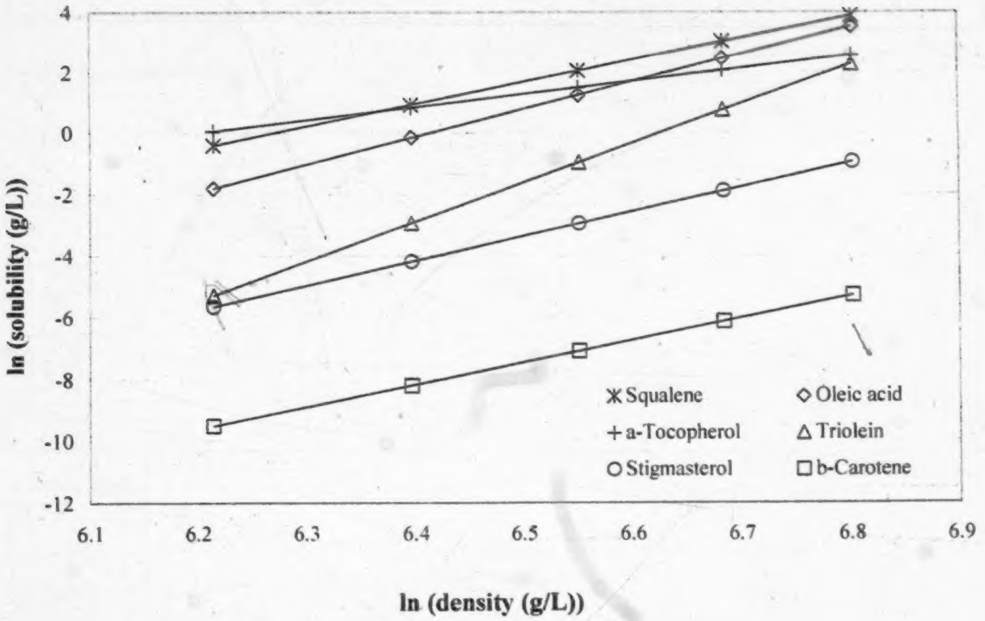

Figure 3.6. Solubility isotherms of lipid components at 323 K plotted using model parameters estimated using equation 2.2 (Tables 2.3. and 3.4).

cholesterol and stigmasterol suggesting similar attractive interaction constants for these sterols. As the differences between solute properties increase molecular weight fails to be an indicator of solubility and other factors that might contribute to interactions should also be considered. For example, in laurin and olein glyceride series, both polarity and molecular weight affected the solubility behavior where the dominant effect was observed to be dependent on CO_2 density (Chapter 2).

Squalene was the most soluble component of all the studied compounds (Fig. 3.6). Despite its higher molecular weight (C30) it was more soluble than oleic acid (C18) probably due to its hydrocarbon or non-polar nature. The solubility of α-tocopherol was similar to that of squalene at lower CO_2 densities. However, the low R^2 values obtained for α-tocopherol should be noted. Stigmasterol was less soluble than triolein, while β-carotene was the least soluble of all the solutes studied throughout the whole CO_2 density range.

Data of Johannsen and Brunner [119] show similar solubilities for δ-tocopherol and α-tocopherol at 353 K whereas a crossover of isotherms was observed at 313 and 333 K at pressures of 28 and 22 MPa, respectively, such that α-tocopherol solubility was higher at lower pressures whereas δ-tocopherol was more soluble at higher pressures. The fact that a lower purity δ-tocopherol sample (90%) was used should be noted and the impact this may have on solubility should not be overlooked.

3.3.3. Implications for supercritical fluid extraction and fractionation processes

The study of the effect of operating conditions on the solubility behavior of minor lipid components provides valuable information that can be used for the design of processes targeting extraction and fractionation of these components from natural sources and the interpretation of the data obtained from such processes. However, the effect of operating conditions on all aspects/mechanisms of the extraction/fractionation as well as the location of the components of interest in the solid matrix of natural materials should be considered while studying process behavior.

SFE kinetics is made up of three periods as discussed in Section. 1.2.2.3.1. During SFE of palm oil from palm shell and pulp, de Franca et al. [150] obtained fractions with high carotene levels only in the diffusion-controlled period and concluded that carotenes were expected to be found in the oleaginous fractions located deep inside the solid particles. However, even in the extraction of pressed palm oil fibers [151], where no diffusion is involved, the shape of the extraction curve may resemble curves with a diffusion controlled period due to the dynamic nature of extract composition, which results from the differences in the solubilities of the extract components.

Natural extracts are a mixture of a large number of compounds. For example, the extracts obtained in the extraction of carotenoids from natural sources can be a mixture of carotenoids (β-carotene and lutein), β-carotene isomers (*cis* and *trans*) or β-carotene and oil; similarly, tocopherol extracts can be a mixture of different tocopherol isomers in an oil base. Some extracts can be mixtures of minor and major lipid components such as vegetable oils. The dynamic nature of extracts can be used to affect separation as in fractional extraction where fractions are collected as a function of time throughout the extraction. In such complex mixtures, the presence of other solutes may affect the solubility of the component of interest. For example, vegetable oil present in the extract may act as a cosolvent and increase the solubility of β-carotene [121] or the presence of *cis* isomers of β-carotene in algae extracts may increase *trans* isomer solubility [125].

Extraction kinetics of the components of interest could be determined by analysis of individual extract fractions throughout the extraction. The effect of operating conditions on binary solubility behavior has been discussed in previous sections. Temperature and pressure also affect extraction behavior through their effect on mass transfer kinetics. Binary diffusion coefficients decrease with pressure and increase with temperature [152, 153]. During the extraction of vitamin E from wheat germ oil, Ge et al. [154] observed a crossover of Vitamin E yield isotherms with time during the course of the extraction at 27.6 MPa such that during the first 45 min, yield decreased with temperature, whereas after 50 min, yield increased slightly with temperature resulting in similar yields at the end of the extraction (120 min). This crossover was attributed to the competing effects of temperature on density/solubility and mass transfer.

In order to determine the effect of operating conditions on loading/solubility of extract from a natural matrix it is necessary to determine solubility from constant extraction rate data. In most extraction studies however the extraction data are reported as extraction yield/recovery after a certain period of time. Extraction yield would be proportional to solubility for that particular extract only in the constant rate period. If the extraction proceeds past the first period the effects of temperature and pressure on the different aspects of extraction cannot be differentiated when only the extraction yield/recovery achieved at the end of the extraction period is reported. Therefore, an extraction curve should be reported to allow the readers to follow the course of the extraction.

In general, extraction yield increased with pressure [89, 91, 102] due to the increase in solubility. Vitamin E yield in wheat germ oil extraction increased with pressure, but not linearly, resulting in an optimal pressure range of 27.6-34.5 MPa at 313 K [154]. A solubility maximum was observed for carotene yield from carrots at 50.5 MPa at 313 K [96], which can be attributed to the solubility maxima observed for β-carotene at high pressures. A solubility maximum can also occur due to the differences in the pressure dependence of mixture components or due to the effect of pressure on diffusion. Vega et al. [93] reported no pressure effect on the extraction/recovery of carotene from carrots.

Another important factor that has a significant impact on the extraction of natural matrices is sample pretreatment. The effects of pretreatment on the efficiency of $SCCO_2$ extraction of oils from oilseeds have been well established as discussed in Chapter 1. Sample pretreatment, such as flaking, which increases the surface area and ruptures cell walls improves oil recovery considerably. Moisture content of the samples may increase extraction efficiency due to swelling of tissues; however, presence of water may also hamper the extraction by decreasing the contact of solvent and the solute. The increase in extraction yield of vitamin E from wheat germ with decreasing moisture content from 11.5 to 5.1% was attributed to improved penetration of $SCCO_2$ into wheat germ tissues. However, a further decrease in moisture content to 4.3% decreased the yield due to shrinking of cell structure [154]. Extraction yield of carotenoids from sweet potatoes was increased by decreasing the moisture content and particle size of the samples [96]. A drying method that minimizes sample degradation such as freeze drying and grinding is carried out prior to extraction of carotenoids for moisture removal and particle size reduction, respectively [89, 94]. Goto et al. [95] reported lower extraction efficiency of carotenoids from freeze dried samples compared to fresh samples and concluded that cell disruption was essential only for freeze dried samples. Pressure effects were observed to be dependent on sample pretreatment such that pressure increased the extraction rate of carotenes from raw carrots whereas pressure did not have any effect on the extraction of freeze dried carrots [95].

The effect of temperature on extraction yield was dependent on the range under investigation. Temperature had a significant effect on β-carotene yield from tomato paste waste at 30 MPa from 308-318 K but not from 318-328 K [102]. Extraction yield of β-carotene from ripe tomatoes at 27.58 MPa increased with temperature from 313-333 K but did not change in the temperature range of 333-353 K [100]. Extraction yield of carotene from *Dunaliella salina* decreased with temperature at 20 MPa in the temperature range of 313-333 K [98]. Spanos et al. [89] reported a crossover between the extraction yield isotherms of sweet potato carotenoids (90% β-carotene) at 27.6 MPa in the temperature range of 311-321 K. Crossover of vitamin E yield isotherms was observed at 20.6 MPa in the temperature range of 308-323 K [154]. The reported crossover pressures based on extraction studies are higher and lower than those observed for pure β-carotene and α-tocopherol solubilities, respectively (as discussed in previous sections). This deviation may be due to a number of reasons: the multicomponent nature of the extracts, the difficulty in the accurate determination of crossover pressures and the use of extraction yields rather than solubility data in the determinations.

Information on relative solubilities and the effect of operating parameters on the extraction behavior of components of interest can also be used in the design of fractionation processes. For example, significant differences in the solubilities of tomato skin carotenes [155], different temperature dependence of lycopene and β-carotene [100, 102] and different pressure dependence of lutein and β-carotene [91] can be exploited for fractionation purposes. Using a two-step process (27.6 MPa at 313 K and 353 K), Cadoni et al. [100] obtained an extract containing 87% lycopene and 13% carotene from tomatoes. King et al. [156] could not achieve any isolation of tocopherols from soybean and wheat germ oils at 25 MPa and 313 K, whereas rice bran oil was preferentially extracted relative to tocopherols. Increasing the temperature to 353 K led to enhancement in the recovery of tocopherols relative to oil, mostly for soybean oil due to different temperature dependence of TG and tocopherol solubility. Shen et al. [157] studied the extraction of minor oil components such as tocopherol, oryzanol and sterols in addition to FFA and TG during the pilot scale extraction of rice bran oil. While the extraction curves of tocopherol and sterols were similar to that of rice bran oil, extraction curves of oryzanol became steeper or remained constant as extraction progressed. The greatest difference among partition coefficients of all components were observed at 17 MPa and 313 K where partition coefficient of FFA was 3.8 times that of TG, 3.19 times that of α-tocopherol, and 11.52 times that of oryzanol.

3.4. SUMMARY

Minor lipid components have gained importance in recent years due to increasing evidence on their health benefits. Consumer demand for natural products and mild conditions required for the processing of these sensitive compounds make $SCCO_2$ technology an attractive alternative to traditional processes. Successful application of this technology to the processing of minor lipid components requires a fundamental understanding of the physical and chemical properties as well as the solubility behavior of these components.

Binary solubility behavior of minor lipid components in $SCCO_2$ was studied to determine the general trends of solubility behavior as affected by solute properties and operating conditions. Solubility behavior of β-carotene and α-tocopherol in $SCCO_2$ has been widely investigated. However, there are wide discrepancies between the reported results arising from differences in solute purity and experimental techniques used. Extreme care must be taken during solubility measurements to eliminate possible sources of variation such as sample degradation. There are few data available for squalene and sterols, whereas the solubility behavior of tocotrienols in $SCCO_2$ has not been reported.

Solubility increased with pressure for all solutes while a solubility maximum was observed for β-carotene and β-sitosterol at high pressures. The temperature dependence of solubility was dependent on pressure and temperature. The investigated range should be noted while reporting the effect of operating conditions on solubility behavior. The effects of solute properties on solubility behavior and relative solubilities of lipid components could be determined by comparing the binary solubility behavior. While vapor pressure/molecular weight is the determining factor in homologous series, differences in intermolecular interactions resulting from polarity differences should be considered for other solutes.

Differences in solubilities and in the effect of operating conditions on solubility behaviors can be used to affect separation of lipid components. Study of binary systems provides valuable information on the fundamentals of solubility behavior and the basis for the study of complex multicomponent mixtures. However, further study of multicomponent systems is required for the design of optimal fractionation processes as multicomponent behavior deviates from that predicted by binary data. $SCCO_2$ extracts of natural materials are multicomponent mixtures where the presence of other solutes affects the solubility behavior of the component of interest. The dynamic nature of the extract composition can be used to fractionate the extract components as in fractional extraction. Multicomponent analysis of extract composition can provide valuable information on the extraction kinetics of these solutes that can be exploited for fractionation purposes.

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4. SOLUBILITY BEHAVIOR OF TERNARY SYSTEMS OF LIPIDS, COSOLVENTS AND SUPERCRITICAL CARBON DIOXIDE AND PROCESSING ASPECTS[1]

4.1. INTRODUCTION

Knowledge of solubility behavior of solutes of interest in $SCCO_2$ is required for the design of any supercritical process. Information on the fundamentals of solubility behavior of binary systems as affected by operating conditions and solute properties provide the basis for the study of more complex multicomponent mixtures. A systematic approach to describe the binary solubility behavior of major and minor lipid components in $SCCO_2$ has been presented in Chapters 2 and 3, respectively.

It is well known that the phase behavior of solutes in $SCCO_2$ can be modified by the addition of a small amount of cosolvent. The main effect of a cosolvent is the solubility enhancement that results from an increase in solvent density and/or intermolecular interactions between the cosolvent and the solute. Selectivity of a separation can be improved by cosolvent addition if there are specific intermolecular interactions between the cosolvent and one or more of the mixture components.

Cosolvent addition can thus improve the feasibility of a process by increasing solvent loading and improving selectivity; however, it may also complicate process design and product recovery. Therefore, benefits of cosolvent addition must be balanced against its disadvantages for a specific application. Choice of cosolvent for a specific application requires a good understanding of the effect of cosolvent addition on the solubility behavior, mass transfer and economics of the process. Ethanol is the preferred cosolvent for food applications due to its GRAS (Generally Recognized As Safe) status. Cosolvent effects on solubility, extraction or fractionation behavior of some lipid components have been investigated; however, a systematic investigation of the cosolvent effect on the solubility behavior of major and minor lipid components in $SCCO_2$ and the implications for extraction and fractionation processes has not been carried out. Therefore, the objectives of this study were: a) to review the effect of cosolvent addition on solubility behavior of solutes with special emphasis on lipids, b) to interpret and correlate the cosolvent effects observed for lipid components with a special focus on ethanol, and c) to assess the implications of the findings for processing of fats and oils.

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[1] A version of this chapter is to be submitted to the Journal of Supercritical Fluids for consideration for publication.

4.2. SOLUBILITY DATA AND ANALYSIS

Literature equilibrium solubility data of ternary systems of major and minor lipid components as well as cocoa butter, cosolvents and $SCCO_2$ have been compiled in Table 4.1. Solubility data for vegetable oils in $SCCO_2$ and ethanol mixtures have also been reported in studies focusing on the processing of vegetable oils such as pistachio oil [18], palm oil [19] and sunflower oil [20]. In extraction studies, solubility data were determined from the slope of the linear portion of the extraction curve [18, 20]. Such data for fats and oils were also included in the analysis carried out in this study. Brunner and Peter [21] reported solubility of palm oil at 70 °C in CO_2 + ethanol mixtures; however, the method of data collection was not noted.

The effect of cosolvent addition on the solubility behavior of fatty acids (FA) (stearic, palmitic and behenic acids), squalene and β-carotene was studied by calculating

Table 4.1. Literature solubility data for ternary systems of pure lipids, $SCCO_2$ and cosolvents.

Component	Cosolvent	Cosolvent concentration	T (K)	P (MPa)	Ref.
Fatty acids					
palmitic acid	ethanol	0.99-8.83^a	308	9.9, 19.7	1
	methanol	0, 3^b	313, 323	10.33-24.12	2
	octane	0.66-8.24^a	308	9.9, 19.7	1
	acetone	0, 3^b	313, 323	10.33-24.12	2
	water	0, 3^b	313, 323	10.33-24.12	2
stearic acid	ethanol	0-2.83^a	318	9.8-16.3	3
	ethanol	0-3.93^a	308	8-16	4
	ethanol	0.47-8.75^a	308	9.9-19.7	1
	methanol	0, 3^b	313, 323	10.33-24.12	2
	octane	0-3.96^a	318	9.6-16.5	3
	octane	1.02-8.83^a	308	9.9, 19.7	1
	octane	0-3.01^a	308	8-16	5
	acetic acid	0-3.32^a	308	8-16	5
	acetic acid	0-3.13^a	318	9.6-16.2	6
	methyl acetate	0-5.66^a	318	9.6-15.9	6
	acetone	0, 3^b	313, 323	10.33-24.12	2
	water	0, 3^b	313, 323	10.33-24.12	2
	acetonitrile	0-5.30^a	318	9.5-16.5	7
behenic acid	ethanol	0-6.67^a	308, 318	8-16	8
	octane	0-6.66^a	308, 318	8-16	9
	pentane	0-7.50^a	318, 328	9-16	9
Minor lipid components					
β-carotene	ethanol	0, 1^c	343	22.3-37.4	10
	ethanol	0.30-2.37^c	313-333	15-28	11
	ethanol	1.6, 5.9^b	288-328	10-50	12
	methanol	0, 1^c	343	18.0-37.3	10
	methylene chloride	0, 1^c	343	23.4-37.0	10
	vegetable oil	0.15-0.67^c	313-333	19-28.3	11
stigmasterol	ethanol	3.5^b	308	10.19-35.47	13
	methanol	3.5^b	308	10.14-30.39	13
	methanol	0, 3^b	313, 323	10.33-24.12	2
	acetone	3.5^b	308	10.07-30.4	13
	acetone	0, 3^b	313, 323	10.33-24.12	2
	water	0, 3^b	313, 323	10.33-24.12	2
cholesterol	ethanol	3.5^b	308	10.10-35.43	13
	acetone	0, 3^b	313, 323	10.33-24.12	2
	acetone	3.5^b	308	10.08-27.71	13

Table 4.1. continued

Component	Cosolvent	Cosolvent concentration	T (K)	P (MPa)	Ref.
	acetone	3.5-6[b]	318-338	10-22	14
	methanol	0, 3[b]	313, 323	10.33-24.12	2
	methanol	3.5[b]	308	10.46-27.76	13
	water	0, 3[b]	313, 323	10.33-24.12	2
	hexane	3.5-6[b]	318-338	9-22	14
	ethane	3.5-96.5[b]	308-338	8.5-22	15
squalene	ethanol	4.07-12.04[c]	333	20-27.5	16
Fats and oils					
cocoa butter	ethanol	20-25[c]	333	8-30	17

[a] mol % in the supercritical phase (solute inclusive)
[b] mol % (solute free)
[c] wt % (solute free)

the cosolvent effect, which was quantified as solubility enhancement (the ratio of solubility obtained with cosolvent addition to that without a cosolvent [22, 23]) and by plotting solubility enhancement versus cosolvent concentration graphs.

Binary solubility data of stigmasterol and CO_2 and ternary solubility data of stigmasterol + CO_2 and cosolvents (ethanol, methanol and acetone) at 308 K [13] were correlated using Chrastil's model [24] (eq 2.2, Section 2.2.3), where solvent and solvent + cosolvent mixture densities were used to eliminate the density contribution to the cosolvent effect. The model parameters were estimated using a multivariate regression analysis of the SAS statistical software package [25].

4.3. RESULTS AND DISCUSSION

4.3.1. Effects of cosolvents on solubility behavior

The solubility behavior of a solute in $SCCO_2$ can be modified by using small amounts of cosolvents. Addition of a cosolvent not only results in solubility enhancement but also may affect the temperature/pressure dependence of solubility. Solvent-cosolvent mixtures should be in the supercritical state (completely miscible) at the operating conditions to achieve the desired cosolvent effect. As demonstrated for cocoa butter in CO_2 and ethanol mixtures [17], the cosolvent effect will be low at conditions below the critical point of the solvent mixture due to the low concentration of the cosolvent in the supercritical phase. Therefore, the critical locus for each solvent system should be determined for the concentration range of interest, and the extraction should be carried out above these conditions. Alternatively, visual observation can be carried out to ensure the presence of a single phase at the experimental conditions, provided such equipment is available. The critical loci for binary systems containing CO_2 and cosolvents have been reported for various cosolvents such as acetone [26, 27], n-alcohols [26, 28, 29] and n-alkanes [26]. Phase equilibria of binary systems of CO_2 and cosolvents have been reviewed by Page et al. [30].

4.3.1.1. Solubility enhancement (Cosolvent effect)

Solvent power of a SCF can be increased by the addition of cosolvents. In general, the addition of a cosolvent with a higher critical temperature than the SCF increases the solvent power of the SCF, whereas cosolvents with lower critical temperatures have the opposite effect [31] as demonstrated for atmospheric gases, N_2 or Ar in CO_2 [32].

Increase in solvent density and intermolecular interactions are the major factors that contribute to the cosolvent effect. In multicomponent systems, solubility can be enhanced selectively or non-selectively. For those systems where the increase in solubility is due to an increase in the density of the solvent mixture, the selectivity is not improved. The entrainer effect [33], which is defined as an increase in both solubility and selectivity, is obtained when there are specific intermolecular interactions between the cosolvent and one of the solutes, such as H-bonding.

The addition of cosolvents resulted in solubility enhancement in all the lipid systems investigated in this study, but to different extents. The magnitude of the cosolvent effect was dependent on the cosolvent, solute and operating conditions. The effect of ethanol addition on the solubility of lipids has been summarized in Table 4.2. The effect of these parameters and differences in the solubility enhancements obtained for different solutes is explored further in the following sections.

4.3.1.1.1. Density effect

Literature on the density of CO_2-cosolvent mixtures at high pressures is relatively scarce [34-36]. A comparison of the density isotherms of pure CO_2 and binary mixtures of CO_2 and cosolvents, acetone, ethanol and pentane at 323 K is presented in Figure 4.1. Density of CO_2 + cosolvent is higher than pure CO_2 density at lower pressures; however, a crossover of density isotherms of acetone and ethanol systems is observed at approximately 23 MPa such that density of CO_2 is higher at higher pressures. This crossover also occurs for pentane but at a lower pressure (around 15 MPa). The densities of solvent mixtures containing ethanol and acetone are similar up to approximately 50 MPa whereas at higher pressures ethanol mixture has a slightly higher density. The density of pentane mixture (highest molar volume) is lowest under the investigated conditions.

The addition of a cosolvent increases the bulk density of the SCF due to the higher density of the cosolvent and clustering of SCF molecules around the cosolvent [37]. The density increase is most pronounced near the critical point of the solvent

Table 4.2. Cosolvent effect (solubility enhancement) of ethanol in lipid systems.

Solute	Solubility enhancement[a]	Ethanol concentration	T (K)	P (MPa)	Data from ref.
Fatty acids					
palmitic acid	1.53-63.66[b]	0.99-8.83[e]	308	9.9, 19.7	8
stearic acid	1.2-63.2[b]	0.47-8.75[e]	308, 318	8-19.7	4, 5, 6
behenic acid	2.0-29.2[b]	1.21-6.67[e]	308, 318	8-16	7
Minor lipid components					
β-carotene	2.19-9.83[b]	0.30-2.37[f]	313-333	15-28	8
stigmasterol	4.0[b]	3.5[g]	308	15.2	1
squalene	1.76-5.92[d]	4.07-12.04[f]	333	20-27.5	9
Vegetable oils					
palm oil	20[c]	10[f]	343	20	1
pistachio oil	4.8[c]	10[f]	333	34.5	25

[a] Solubility enhancement = the ratio of solubility obtained with cosolvent addition to that without a cosolvent

[b] Calculations based on solubility in mole fraction

[c] Solubility in wt %

[d] Solubility in w/w

[e] mol % in the supercritical phase (solute inclusive)

[f] wt % (solute free), [g] mol % (solute free)

mixture as the difference between the density of the cosolvent and that of the SCF and clustering are highest near the critical point. The difference between the density of SCF mixture and the SCF decreases with pressure and density isotherms eventually crossover as SCF density increases and clustering decreases with pressure.

Therefore, the effect of cosolvent addition on solvent density, hence density contribution to the cosolvent effect is dependent on the cosolvent and pressure at a given temperature. In order to differentiate the contributions of density and intermolecular interactions to the cosolvent effect, density of the solvent mixture should be determined. Solvent mixture density can be measured or can be calculated using an equation of state [14, 15, 23]. Density contribution to the cosolvent effect can then be calculated as the ratio of the binary solubility based on mixture density and the solubility based on pure CO_2 density at the same pressure and temperature [22, 23]. The density contribution to

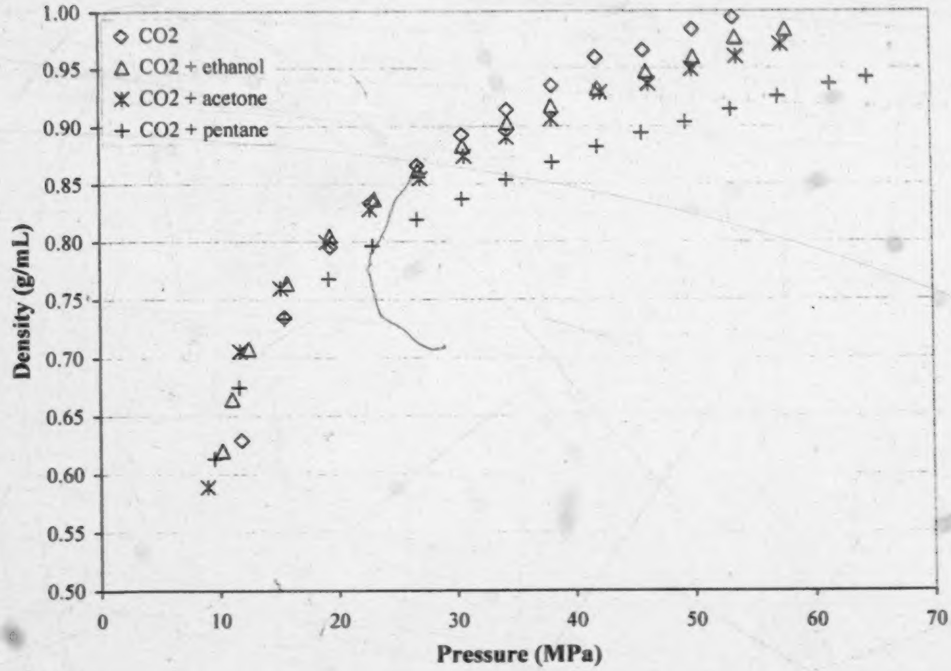

Figure 4.1. Density isotherms of binary mixtures of CO_2 + cosolvents (10% w/w ethanol, 12 % w/w pentane and 10 % w/w acetone) at 323 K (Data from Refs. 34-36).

the solubility enhancement of m-hydroxybenzoic acid (HBA) and o-HBA in $SCCO_2$ and methanol was 1-2% and 4-11% of total cosolvent effect, respectively [23]. The density contribution was observed to decrease with increasing cosolvent concentration for CO_2 + methanol + naproxen system in the cosolvent concentration range of 1.75-5.25% such that at 1.75% methanol, the density increase was responsible for 30-70% of the solubility enhancement at all pressures, whereas the density contribution was not as significant at higher methanol concentrations [22]. When the solubility enhancement observed is solely due to the density effect, binary and ternary solubility isotherms (plotted as a function of solvent mixture density) coincide as observed for cholesterol in supercritical ethane and cosolvents, hexane (1.75-3.5 mol%) and propane (3.5-14 mol%) [14, 15].

Estimated Chrastil's model parameters (Table 4.3) were used to plot solubility isotherms for stigmasterol + cosolvent systems (Fig. 4.2). As the measured densities of CO_2 and CO_2 + cosolvent mixtures were used in the correlation, the effect of cosolvent addition on solvent density is removed and therefore the difference in the isotherms reflect the differences in specific intermolecular interactions. The difference in cosolvent effects of methanol and ethanol is due to the difference in the stoichiometry of the stigmasterol-alcohol complexes formed in the solid phase [13].

4.3.1.1.2. Intermolecular interactions

Physical interactions between the solute and cosolvent such as dipole-dipole, dipole-induced dipole, or induced dipole-induced dipole (dispersion) interactions and specific interactions such as H-bonding and charge transfer complexes are important contributors to the cosolvent effect. Therefore, accurate interpretation of the cosolvent

Table 4.3. Model parameters for stigmasterol in pure CO_2 and CO_2 + cosolvent mixtures at 308 K estimated using equation 2.2 (lnc = k′lnd + b′).

Solvent system	**k′ ± standard error**	**b′ ± standard error**	**R^2**
CO_2	6.9±0.3	-49.1±2.1	0.990
CO_2 + 3.5 mol % ethanol	6.7±0.5	-46.5±2.2	0.991
CO_2 + 3.5 mol % methanol	6.8±0.2	-48.3±1.1	0.995
CO_2 + 3.5 mol % acetone	7.8±0.3	-54.5±2.0	0.991

effect requires knowledge of the intermolecular interactions between the solutes and solvents of interest.

Solvents can be characterized according to their chemical constitution, using physical constants, in terms of acid-base behavior and in terms of specific solute-solvent interactions [38]. Solvent polarity, as a physical property is given by the dipole moment of the solvent. It can also be defined as the overall solvation power of a solvent, which depends on all possible nonspecific and specific intermolecular interactions between the solute and the solvent, excluding interactions leading to chemical alterations of the solute molecules (protonation, oxidation, reduction, chemical complex formation). Solvent polarity defined as such cannot be described by a single physical solvent property such as dielectric constant or dipole moment; however, physical constants can be used to interpret certain intermolecular interactions. For example, the dipole moment of the solvent determines the strength of the dipole-dipole interactions of the solvent, whereas polarizability of the solvent will determine the strength of the dipole-induced dipole, or induced dipole-induced dipole interactions. Empirical solvent parameters such as Kamlet-Taft solvent parameters [39-41] can be used to describe separate contributions of H-bond donor (α) and H-bond acceptor (β) ability, and dipolarity/polarizability (π*) of solvents

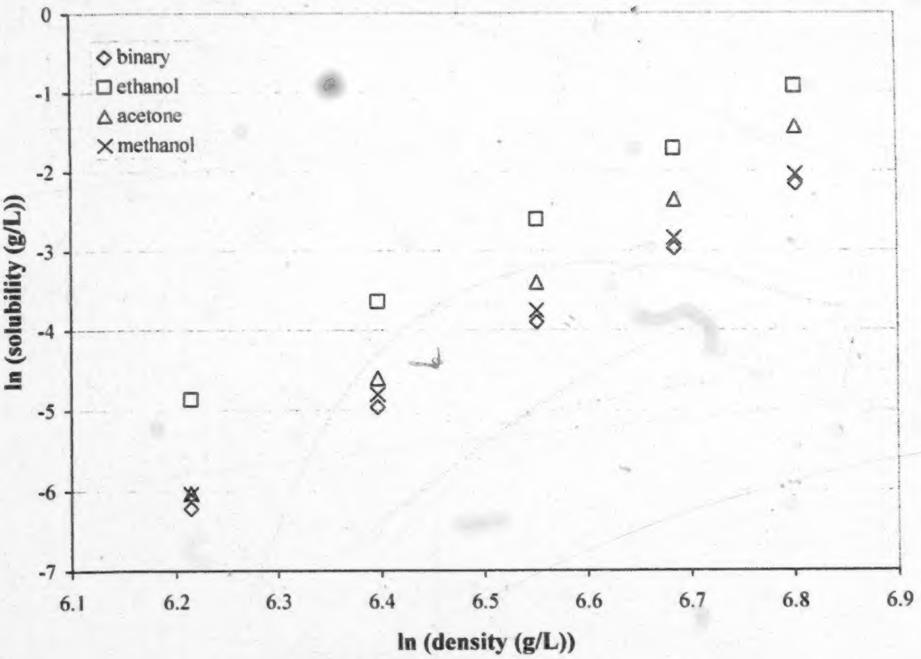

Figure 4.2. Solubility isotherms of stigmasterol at 308 K in pure CO_2, CO_2 + ethanol, CO_2 + methanol and CO_2 + acetone (Data from Ref. 13).

to overall solvent polarity. Physicochemical properties of selected solvents are summarized in Table 4.4.

Because of the symmetry of its molecule, CO_2 does not have a net dipole moment but has a substantial quadrupole moment. Its polarizability is 2.74×10^{-30} m^3 [22]. Lewis acid and base interactions of CO_2 have been studied by various researchers [44-46]. Kamlet-Taft solvent parameters have been used for the characterization of $SCCO_2$. Ikushima et al. [47] reported α values for CO_2 ranging from negative values at densities above 0.44 g/cm^3 to 0.195 at 0.25 g/cm^3. Hyatt [48] reported that CO_2 might be in the ether-ethyl acetate H-bond basicity range; however, Sigman et al. [49] observed negative β values for $SCCO_2$, which were independent of density. Ikushima et al. [47] also observed very small values for Lewis basicity parameter (B_{MeOD}) of $SCCO_2$ (which can be linearly correlated with β), which was comparable to that of hexane and therefore concluded that CO_2 can be regarded as a non H-bond acceptor solvent. Unlike Sigman et al. [49], Ikushima et al. [47] observed that Lewis basicity increased with density. Dipolarity/polarizability (π^*) values for CO_2 were very low [47, 49, 50] and decreased from a value near zero to more negative values as density decreased (approached zero) [47, 49]. Solvent parameters for nearcritical and supercritical ethanol [51] and for $SCCO_2$-n-alcohol mixtures [52] have also been reported.

Intermolecular interactions in pure and mixed SCFs and their role in the cosolvent effect have been investigated using several different solvents, cosolvents and solutes with varying physical and chemical properties [53-59]. Specific intermolecular interactions such as H-bonding and charge transfer complexes formed between the cosolvent and solutes are important contributors to the cosolvent effect and can be used to improve the

Table 4.4. Physicochemical properties of selected cosolvents [42, 43].

Cosolvent	Formula	Kamlet-Taft solvent parameters[a]			MW	Boiling point (°C)	Dipole moment (D)[b]	Dielectric constant	Molar volume (cm^3/mol)	Polarizability (10^{-30} m^3)
		α	β	π^*						
pentane	C_5H_{12}	0	0	-0.15	72	36	0	1.844	115.0	10.2
hexane	C_6H_{14}	0	0	-0.11	86	69	0	1.9	130.5	11.9
octane	C_8H_{18}	0	0	0.01	114	126	0	1.95	163.5	15.6
methanol	$C_1H_4O_1$	0.98	0.66	0.60	32	64	1.7	32.6	40.4	3.3
ethanol	$C_2H_6O_1$	0.86	0.75	0.54	46	78	1.7	22.4	58.68	5.1
methyl acetate	$C_3H_6O_2$	0	0.42	0.49	74	57	1.7	6.7	79.8	7.0
ethyl acetate	$C_4H_8O_2$	0	0.45	0.45	88	77	1.7	6.02	98.5	8.8
acetone	$C_3H_6O_1$	0.08	0.48	0.62	58	56	2.9	20.6	73.4	6.4
acetonitrile	$C_2H_3N_1$	0.19	0.40	0.66	41	81.6	3.2	37.5	52.86	4.4
ether	$C_4H_{10}O_1$	0	0.47	0.24	74	34.5	1.3	4.3	103.5	8.9
acetic acid	$C_2H_4O_2$	1.12	0.45	0.64	60	118	1.7	6.2	57.5	5.2
water	H_2O	1.17	0.47	1.09	18	100	1.87	79.7	18.02	1.5

[a] α: H-bond donor acidity, β: H-bond acceptor basicity, π^*: polarity (dipolarity/polarizability)

[b] For liquids at 298 K.

selectivity of a separation. Complex formation between the solute and cosolvent can lead to large solubility enhancements such as that observed for hydroquinone and tri-n-butyl phosphate in $SCCO_2$ [60]. Cosolvent effects observed in $SCCO_2$-cosolvent mixtures and various solutes have been attributed to H-bonding interactions between the solute and cosolvent [4, 5, 13, 33, 61].

H-bonding is a donor-acceptor interaction specifically involving H-bond donor and acceptor atoms. H-bonds are formed when the electronegativity of the H-bond donor A in an A-H covalent bond is such as to withdraw electrons and leave the proton partially unshielded. To interact with this donor A-H bond, the acceptor B must have lone-pair electrons or polarizable π electrons. Functional groups can act both as acceptors and donors (such as OH in water, alcohol, carboxylic acids), or as acceptor only (such as C=O in carboxylic acids, ketones, esters), or as donor only. Most common H-bonds in nature and chemistry are the moderate H-bonds, which are formed between neutral donor and acceptor groups such as -OH, O=C [62].

H-bonding exists in supercritical methanol [63], ethanol [63, 64] and water [65]. The existence of H-bonding interactions between solutes and cosolvents, and cosolvents and solvents in supercritical systems has also been demonstrated. H-bonding of methanol-d (deuterium labeled methanol) and formation of a weak complex between methanol-d and CO_2 due to quadrupole-dipole interaction have been observed in $SCCO_2$-methanol-d mixtures [66]. Reilly et al. [44] reported that complex formation between methanol-d and liquid CO_2 was due to Lewis acid-base interaction between the carbon atom of CO_2 and the oxygen of methanol rather than H-bonding and concluded that CO_2 molecule was more likely to accept an electron pair than donate one.

H-bonding of benzoic and salicylic acids in $SCCO_2$ with methanol and ethanol [67], acetic and palmitic acids in $SCCO_2$ with ethanol [68] and water [56] and stearic acid with ethanol [4, 69], dimethyl sulfoxide [69] and acetonitrile [69] has been demonstrated using FT-IR spectroscopy. Carboxylic acids interacted more readily with ethanol than water, which was attributed to self-association of water molecules [56]. The strength of H-bonding between stearic acid and cosolvents was in the order dimethyl sulfoxide>ethanol>acetonitrile [69]. The extent of H-bonding between the solutes and cosolvents was observed to increase with cosolvent concentration [4, 56, 67-69].

In cosolvent-solvent mixtures, H-bonding between the solute and solvent, solvent and cosolvent, and cosolvent and cosolvent may compete with cosolvent-solute H-bonding and tie up H-bonding sites that might otherwise be available for solvation between solute and cosolvent [70].

In addition to solvent properties, information on the properties of the solutes is also required to better understand the intermolecular interactions involved in the cosolvent systems. Solvatochromic parameters are available for a variety of solutes [71] and are used in linear solvation energy relationships (LSER), which relate a solvation equilibrium property to a linear combination of solute-solvent interaction and cavity-formation parameters (which account for the two steps involved in the solvation process: formation of cavity to accommodate the solute and solute-solvent interactions). LSER has been used for the modeling of solute solubility in $SCCO_2$ and R134a (1,1,1,2-tetrafluoroethane) [72, 73], partitioning of solutes between SCFs and water [74, 75] and to study the intermolecular interactions and retention in SCF chromatography [76].

Available solvatochromic solute parameters of studied lipids are summarized in Table 4.5.

Alessi et al. [77] studied characterization of tocopherol isomers α, δ and γ and tocopheryl acetate, in terms of dipolarity/polarizability, hydrogen bond basicity/acidity, and lipophilicity using an equation of solvation. The dipolarity of acetate was quite higher than that of tocopherols, which was very low, whereas the tendency to interact with polarizable solutes was similar for all. The H-bond acidity of tocopherols followed the order: δ>γ>α>α-acetate. The lower acidity of acetate was explained by the presence of the CH_3COO group instead of the phenolic OH. Low acidity of α-tocopherol has been attributed to steric effects (the presence of methyl substituents ortho to phenolic OH group), which lead to shielding of the phenol group [77, 78]. The H-bond basicity was highest for δ-tocopherol followed by acetate and γ- and α-tocopherols [77]. β-Carotene has been used as an indicator in solvatochromic studies [79, 80] where solute-solvent interactions were mainly due to solvent dipole-solute induced dipole and dispersion forces, whereas H-bonding interactions involving β-carotene as a hydrogen bond acceptor were not very large.

4. 3.1.1.3. Effect of supercritical fluid

The cosolvent effect is also dependent on the nature of the supercritical solvent as the interactions between the solvent-solute and/or solvent-cosolvent can have an impact on the cosolvent-solute interactions and the resulting solubility enhancement. Addition of an alkane cosolvent, like hexane, to ethane did not cause a significant solubility enhancement of cholesterol due to similar cosolvent-solute and solvent-solute interactions, whereas the addition of hexane to CO_2 resulted in a large enhancement as

Table 4.5. Available solvatochromic parameters of lipid components [71, 73].

Solute	**Solvatochromic solute parameters**[a]				
	R_2	**π_2^H**	**$\Sigma\alpha_2^H$**	**$\Sigma\beta_2^H$**	**V_x**
palmitic acid	0.035	0.60	0.60	0.45	2.437
stearic acid	0.015	0.60	0.60	0.45	2.719
triolein	0.08	0.60	0.0	-	8.243
squalene	0.90	0.48	0.0	-	4.078

[a] R_2: an excess index of refraction (R_2=0 by definition for n-alkanes)
π_2^H: solute dipolarity/polarizability
$\Sigma\alpha_2^H$: the effective hydrogen bond acidity
$\Sigma\beta_2^H$: the effective hydrogen bond basicity
V_x: McGowan's intrinsic volume for the solute

cosolvent-solute interactions were stronger than solvent-solute interactions [14]. CO_2 can also compete with the solute or cosolvent to form hydrogen bonds with the cosolvent or solute. The weaker hydrogen bonding ability of CO_2 may be compensated by its higher concentration masking the cosolvent effect [33].

Comparison of solubilities in different SCFs can provide valuable information on the nature of interactions involved in the solvation process. Cholesterol, which has a large hydrocarbon skeleton and a single polar functional group (OH), has a higher solubility in supercritical ethane than CO_2 [14], indicating that dispersion interactions between ethane and cholesterol are stronger than the dipole-quadrupole interactions between CO_2 and cholesterol. Similarly, the solubilities of β-carotene [81], α-tocopherol [82], fatty acid esters [83], triglycerides [84] and vegetables oils [21] were observed to be higher in alkane SCFs such as ethane or propane.

4.3.1.1.4. Effect of cosolvent

Cosolvent effect of non-polar cosolvents such as hydrocarbons (which have higher polarizability than CO_2, except methane) in $SCCO_2$ is due to non-specific dispersion and induced dipole-dipole interactions between the solute and cosolvent. Addition of a hydrocarbon cosolvent with a high molar volume such as octane may increase the solvent strength of the SCF although it decreases the molar density of the solvent [55]. Specific interactions such as H-bonding and dipole interactions between the cosolvent and the solute contribute to the cosolvent effect of polar cosolvents.

Similar cosolvent effects of acetone and ethyl acetate on naproxen in $SCCO_2$ could be indicative of dominant H-bonding interactions in these systems or cancellation of different effects as these cosolvents have similar H-bonding properties, whereas their dipole moments, dielectric constants, molecular sizes, and critical properties are different (Table 4.4) [22]. Higher cosolvent effect of alcohols than acetone and ethyl acetate on naproxen was attributed to the amphiprotic nature of alcohols (ability to act both as a proton donor and as an acceptor), which increase the number of ways they can interact with the solute [22]. The higher cosolvent effect of methanol on benzoic acid was explained by the higher basicity of methanol compared to acetone, whereas similar effects of octane and acetone on benzoic acid in $SCCO_2$ were attributed to large dispersion effect of octane [59].

A comparison of the cosolvent effects of ethanol, octane, acetic acid, methyl acetate and acetonitrile on the solubility of stearic acid in $SCCO_2$ is given in Figure 4.3. Stearic acid is capable of participating in H-bonding interactions both as a H-bond acceptor and as a donor due to its carboxyl group. Ethanol and acetic acid also have both H-bond donor and acceptor properties but to different extents as reflected in their acidity and basicity parameters (Table 4.4), whereas methyl acetate and acetonitrile participate in H-bonding with stearic acid as hydrogen bond acceptors. Octane does not have any H-

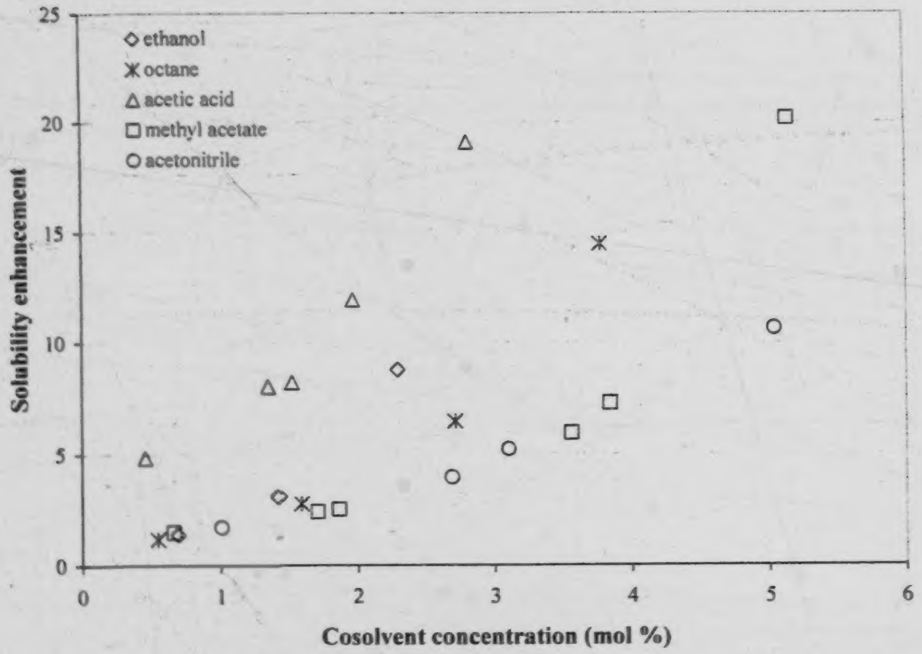

Figure 4.3. Solubility enhancement of stearic acid in CO_2 + cosolvent (ethanol, octane, acetic acid, acetonitrile and methyl acetate) mixtures at 318 K and 9.5-9.8 MPa (Data from Refs. 3, 6, 7).

bond donor or acceptor properties but has the highest polarizability. Solubility enhancement of stearic acid was highest for acetic acid, which has the highest H-bond donor acidity followed by ethanol, suggesting that H-bonding interactions played a significant role in the observed cosolvent effects. Cosolvent effect of octane was higher than those of methyl acetate and acetonitrile due to its higher polarizability. The solubility enhancement in the presence of octane was due to induced dipole-dipole and dispersion interactions. A higher cosolvent effect for ethanol has also been observed for palmitic, stearic and behenic acids in $SCCO_2$ [1, 3, 9] compared to octane. Cosolvent effect of octane on behenic acid was higher than that of pentane [9]. Dobbs et al. [55] also reported that the solubility enhancement increased with chain length or polarizability of the non-polar n-alkane cosolvents indicating that the increase in attractive forces with chain length more than compensated for the increase in repulsive forces.

A higher solubility enhancement of β-carotene was observed in CO_2 + ethanol (1 wt%) than in CO_2 + methanol (1 wt%) mixtures [10], which is consistent with β-carotene solubility in liquid ethanol and methanol [85]. Solubility enhancement of β-carotene can also be obtained by using vegetable oil as a cosolvent but to a lesser extent than ethanol [10]. It should be noted that under practical operating conditions vegetable oil will not be miscible with $SCCO_2$; therefore, its effect as a cosolvent will be limited by its low solubility in the supercritical phase.

In their study on the analytical supercritical fluid extraction (SFE) of carotenes, Marsili and Callahan [86] studied the influence of cosolvents on the solubility of crystalline β-carotene. While hexane did not increase β-carotene solubility significantly, the addition of methylene chloride and ethanol resulted in significant solubility enhancement; however, methylene chloride also caused significant degradation. In $SCCO_2$ extraction of carotenes from carrots, a higher extraction yield was obtained using chloroform compared to hexane [87].

Wong and Johnston [13] studied the solubility behavior of cholesterol and stigmasterol in $SCCO_2$ with cosolvents, acetone, ethanol and methanol (3.5 mol %) at 308 K. Solubility enhancement of cholesterol was highest for methanol followed by acetone and ethanol, whereas the highest enhancement for stigmasterol was observed for ethanol followed by acetone and methanol. The same trends persisted when the effect of density increase on stigmasterol solubility was removed (Fig. 4.2). Formation of complexes between sterols and organic liquid solvents should be considered while interpreting the cosolvent effects for sterols as complex formation between stigmasterol and cosolvent can decrease the solubility hence the cosolvent effect. Solid complexes were formed between cholesterol and ethanol as well as stigmasterol and methanol and ethanol in CO_2 with different stoichiometries [13].

The effect of water addition on the solubility of lipid components in $SCCO_2$ has also been investigated [2, 86]. While the solubility of stigmasterol, cholesterol and FA decreased (at 313, 323 K and 10.33-24.12 MPa, 3 mol % water) [2], solubility of β-carotene (at 313 K and 34.3 MPa) was not affected [86]. It should be noted that under these conditions, water and CO_2 would not be miscible [30], resulting in lower water concentrations in $SCCO_2$ than the reported feed concentrations. Moisture content of natural materials may also affect/modify the solubility behavior of solutes of interest during supercritical processing. Coextraction of water has been reported during $SCCO_2$ oil extraction from various sources such as canola flakes [88] and Atlantic mackerel [89] and was affected by the operating conditions and initial moisture content of the sample. Moisture levels ≥40.5% resulted in significant decrease in the solubility of Atlantic mackerel oil in $SCCO_2$ [89].

The cosolvent effect increased with cosolvent concentration in $SCCO_2$ (up to 12 mol %) for all the investigated lipid systems (Figs. 4.3-4.10). A similar increase in cosolvent effect with cosolvent concentration has been observed for various systems [22, 53]. Non-linear cosolvent effect in cosolvent concentration was typical of the behavior of systems with strong specific interactions [53]. The cosolvent effect was reported to be a weak function of concentration for systems where the cosolvent effect was only due to density increase such as in hexane + supercritical ethane [14, 53]. A decrease in the cosolvent effect or in the rate of increase of cosolvent effect of self-associating cosolvents (cosolvents with both H-bond donor and acceptor properties like alcohols and acetic acid) may occur at high cosolvent concentrations. Such a decrease was not observed in the cosolvent effect of ethanol and acetic acid on stearic acid at 318 K and 9.6-9.8 MPa (Fig. 4.3); however, the rate of increase of cosolvent effect of acetic acid on stearic acid was observed to decrease with cosolvent concentration at pressures higher than 8 MPa at 308 K (Fig. 4.4) implying that self-association of acetic acid had a significant effect on the solubility enhancement under these conditions.

The cosolvent concentration has been reported as the initial concentration fed into the system as mol % or wt % on a solute-free basis [2, 10-17] or as mole fraction of the cosolvent in the supercritical phase on a solute-inclusive basis [1, 3-9]. The concentration of the cosolvent in the supercritical phase will deviate from the initial concentration and be dependent on operating conditions as it is affected by the solubility of the cosolvent

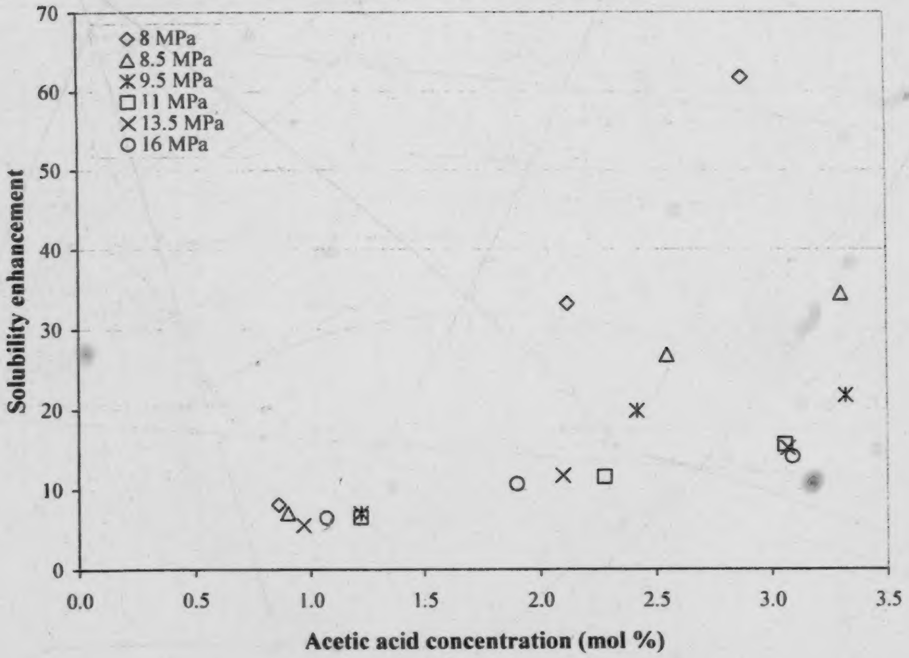

Figure 4.4. Solubility enhancement of stearic acid in CO_2 + acetic acid at 308 K (Data from Ref. 6).

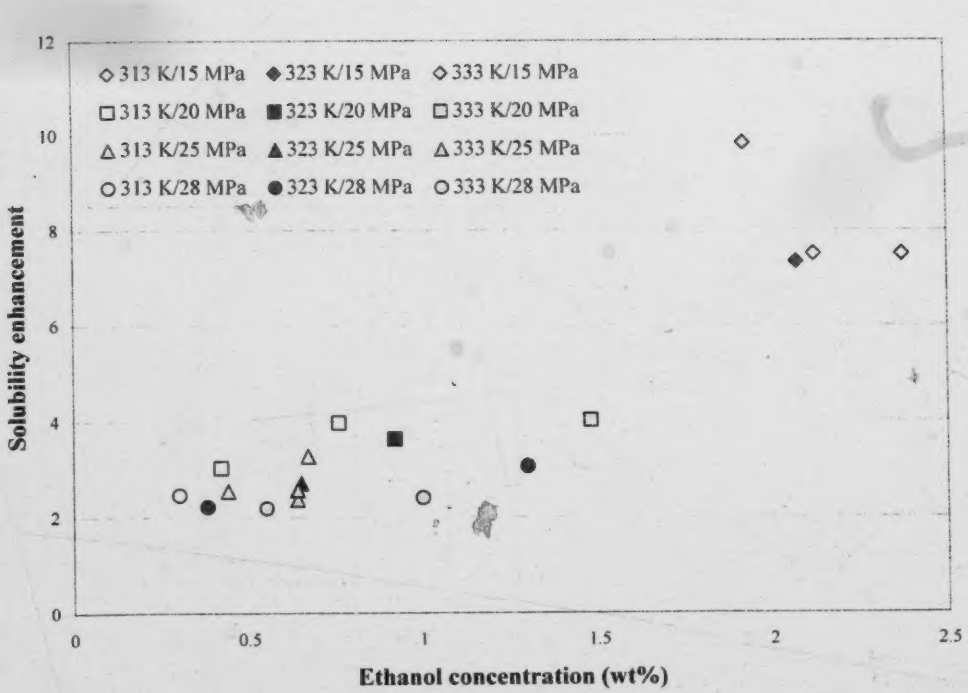

Figure 4.5. Solubility enhancement of β-carotene in CO_2 + ethanol (Data from Ref.11).

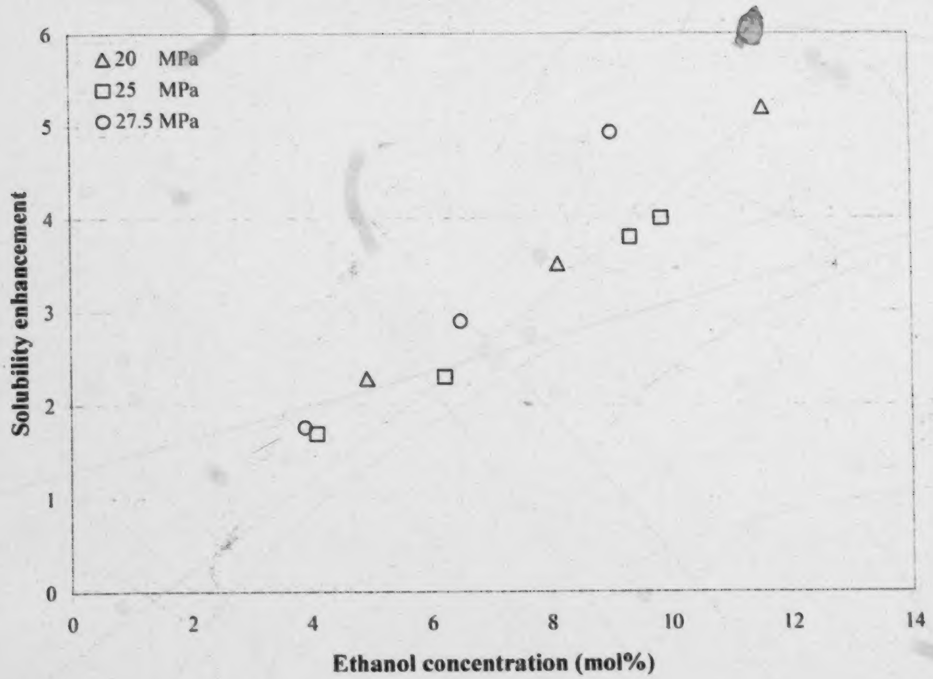

Figure 4.6. Solubility enhancement of squalene in CO_2 + ethanol at 333 K (Data from Ref. 16).

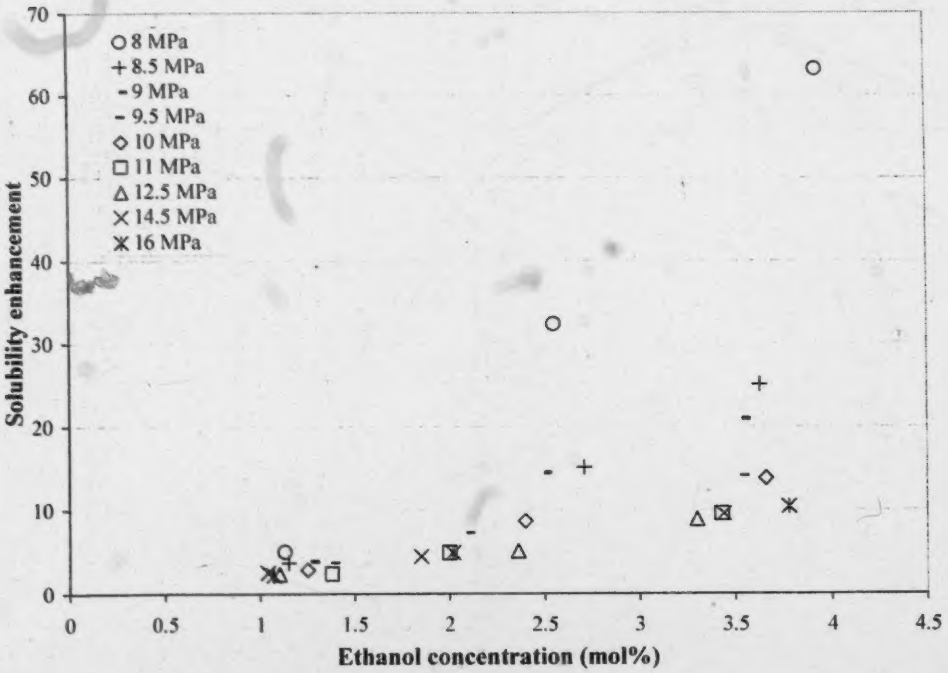

Figure 4.7. Solubility enhancement of stearic acid in CO_2 + ethanol at 308 K (1-3.9 mol% ethanol) (Data from Ref. 4).

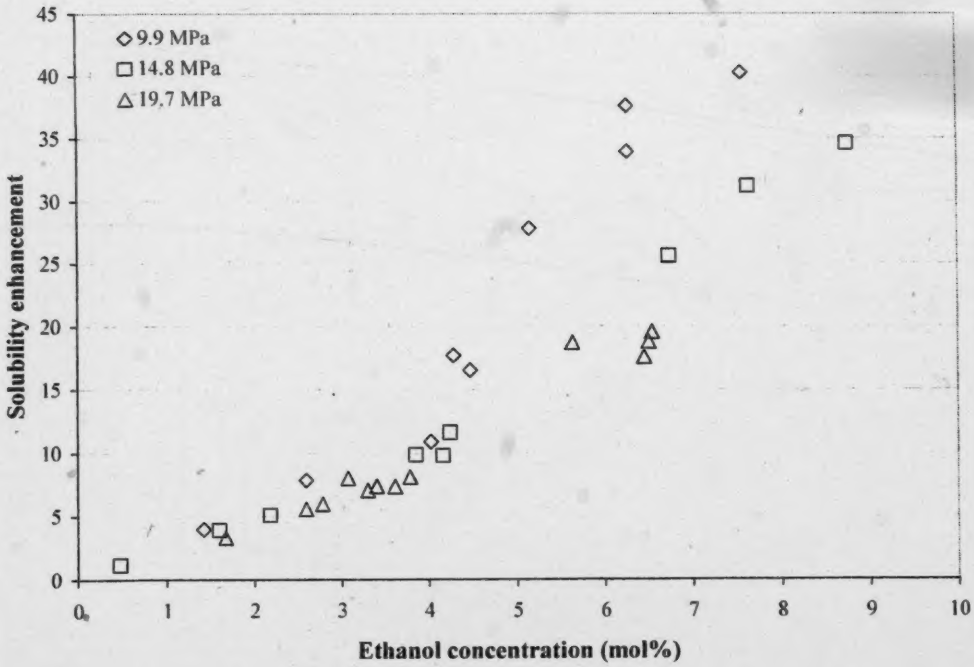

Figure 4.8. Solubility enhancement of stearic acid in CO_2 + ethanol at 308 K (0.5-8.8 mol% ethanol) (Data from Ref. 1).

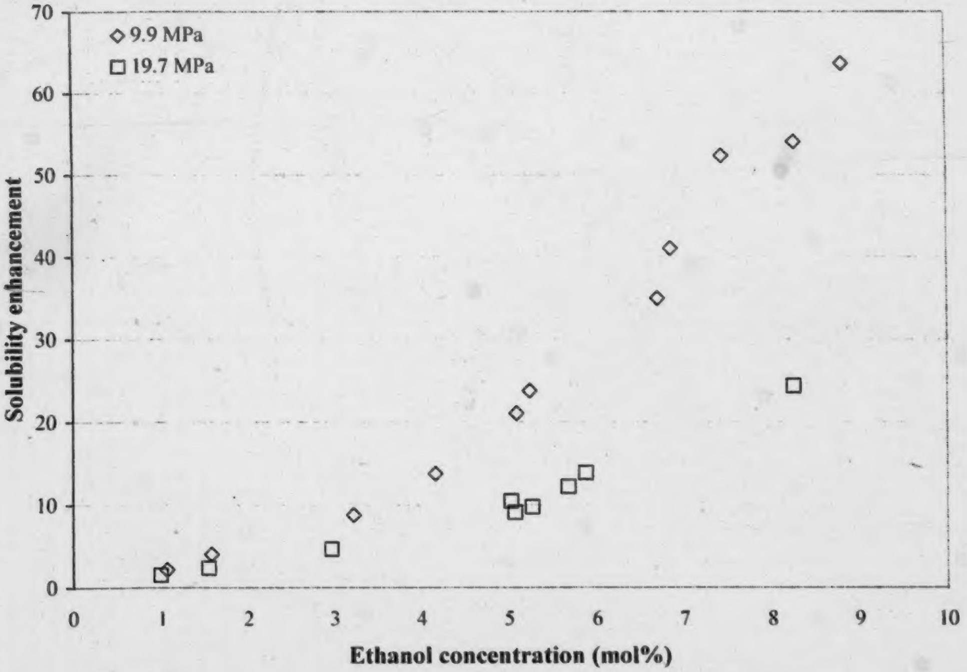

Figure 4.9. Solubility enhancement of palmitic acid in CO_2 + ethanol at 308 K (Data from Ref.1).

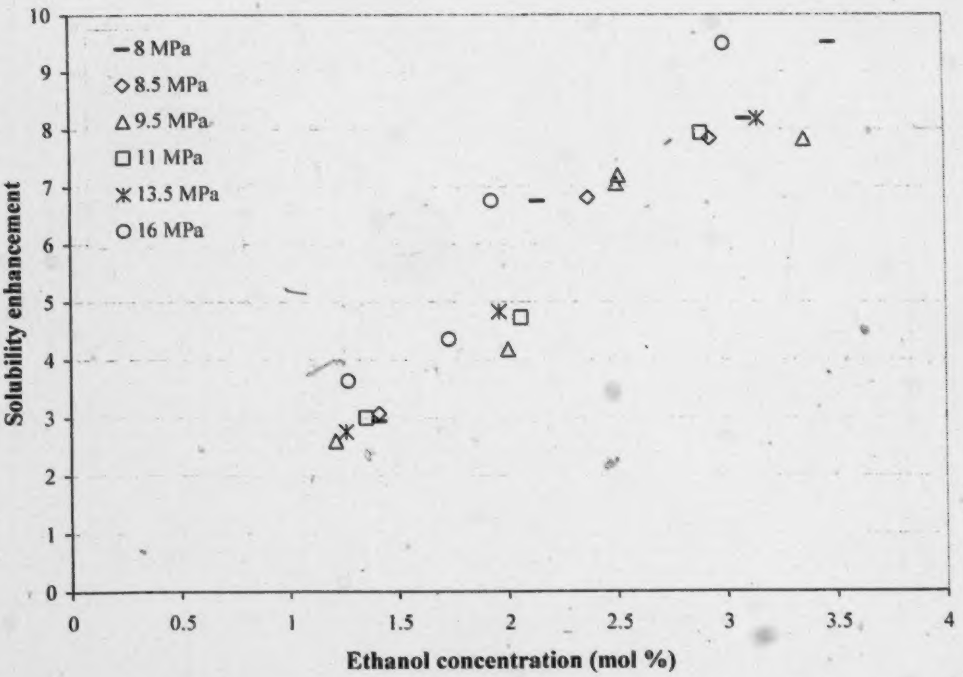

Figure 4.10. Solubility enhancement of behenic acid in CO_2 + ethanol at 308 K (Data from Ref. 8).

and the SCF in the solute and the solubility of the solute in the solvent mixture. This deviation will not affect the observed trends in solubility behavior with cosolvent concentration; however, it should be considered while interpreting the absolute values for the cosolvent effect and the pressure effect as well as when comparing data from different studies. It should be noted that there might be a significant difference between initial cosolvent concentration and concentration in the supercritical phase if the cosolvent and SCF are not miscible at the investigated conditions.

4.3.1.1.5. Effect of solute

The differences in the cosolvent effect on different solutes arise from differences in the intermolecular interactions between the cosolvent and solutes. A polar cosolvent may increase markedly the solubility of a polar solute, but may not affect that of a nonpolar solute. For example, the addition of 3.5 mol % methanol, which has a similar polarizability to CO_2, had a minor effect on the solubility of non-polar hexamethylbenzene, whereas it increased the solubility of 2-aminobenzoic acid up to 650% [59].

Cosolvent effect of ethanol on some lipid components is summarized in Table 4.2. The highest enhancements were observed for stearic and palmitic acids at the highest cosolvent concentrations used, whereas the lowest enhancement was observed for squalene. Tocopherols (-OH and -O-), sterols (-OH), FA (-COOH) and vegetable oils (-COOR) have H-bonding ability; therefore, H-bonding between ethanol and these solutes contributes to the cosolvent effect, whereas squalene and β-carotene mainly interact through induced dipole-dipole and dispersion forces. Cosolvent effects of ethanol on palmitic and stearic acids were similar under the experimental conditions investigated

(Table 4.2); however, Noh et al. [2] reported significantly higher cosolvent effects for methanol and acetone on palmitic acid than on stearic acid (42.9 and 21.5 times higher for methanol and acetone, respectively, at 323 K), which might be due to the differences in the effect of these cosolvents on the melting behavior of these FA under the conditions of the study. Differences in the cosolvent effects of various cosolvents on lipid components can be exploited to achieve fractionation of complex lipid mixtures as discussed in Section 4.3.3.2.

4.3.1.2. Temperature/pressure dependence of solubility

Addition of a cosolvent also affects the temperature and pressure dependence of solubility. The addition of a cosolvent can increase the temperature dependence of solubility as demonstrated for the system hexadecanol and octadecane in CO_2 + hexane (at 11.6 MPa) [90]. The ability to operate at a lower pressure, hence closer to the critical point of the solvent mixture, resulting from cosolvent addition would accentuate the effect of cosolvent on temperature dependence of solubility as the effect of operating conditions on solubility behavior is maximal near the critical point. The effect of temperature on solubility of sunflower oil varied with ethanol concentration such that the change in oil solubility with temperature was higher at 10 wt% than at 5 wt% ethanol [20]. High temperature dependence of solubility observed in cosolvent systems such as palm oil in CO_2 + ethanol (10 wt%) has been proposed as a means to increase the efficiency of solvent regeneration [21]. The effect of cosolvent addition on solvent regeneration will be discussed in Section 4.3.3.3.

For systems where the pressure dependence of solubility is not affected by cosolvent addition, the cosolvent effect would be independent of pressure resulting in similar shapes for binary and ternary solubility isotherms [22, 59]. Decreasing cosolvent effect with pressure is indicative of a lower pressure dependence for the cosolvent system, whereas a higher pressure dependence will result in increasing cosolvent effect with pressure.

The pressure dependence of the cosolvent effect of ethanol on lipid systems was system dependent. Conclusions on the effect of pressure on solubility enhancement could not be drawn for β-carotene as data were not available over the entire pressure range (15-28 MPa); however, the available data showed a decreasing trend with pressure (Fig. 4.5). The solubility enhancement of squalene appeared to be independent of pressure in the pressure range investigated (20-27.5 MPa) (Fig. 4.6). A decrease in the cosolvent effect with pressure was observed for palmitic and stearic acids (Fig. 4.7-4.9). The influence of pressure on the solubility enhancement of FA was dependent on pressure and cosolvent concentration such that it increased with concentration and decreased with pressure. At 308 K (Fig. 4.7), the solubility enhancement of stearic acid in CO_2 + ethanol decreased with increasing pressure in the pressure range of 8-16 MPa. The influence of pressure was more dominant at lower pressures, such that the highest decrease was observed from 8 to 8.5 MPa, whereas the enhancements were similar in the range of 11-16 MPa. A clear trend with pressure cannot be established for behenic acid (Fig. 4.10).

A decrease in cosolvent effect with pressure at constant cosolvent concentration has been reported for various solutes in CO_2 + cosolvent mixtures and has been attributed to the decrease in the contribution of density to the cosolvent effect with pressure [53] and the decrease in local composition enhancement with pressure [22, 23]. The local concentration of the cosolvent around the solute is higher than the bulk composition, especially near the critical point due to the clustering of cosolvent molecules around the solute. However, this composition enhancement decreases with pressure as the local concentration approaches the bulk concentration at high enough pressures [22, 23]. Data of Koga et al. [1] show that the solubility enhancement for stearic acid was independent of pressure (9.9-19.7 MPa) up to an ethanol concentration of around 4.3 mol %, whereas at higher concentrations the enhancement decreased with increasing pressure (Fig. 4.8). Pressure independence of cosolvent effect at low cosolvent concentrations was also observed in the naproxen-CO_2-cosolvent systems [22], where it was attributed to the dominant effect of absolute local concentration of cosolvent (which increases with pressure) on cosolvent effect at low cosolvent concentrations. The effect of local composition enhancement became apparent with increasing cosolvent concentration and therefore a decrease in cosolvent effect was observed with pressure [22]. Cosolvent concentration in solvent mixtures that are not in the supercritical state is expected to increase with pressure due to increasing solubility of cosolvent in the supercritical phase. However, in such cases the phase equilibria can get complicated due to the formation of additional phases.

An increase in cosolvent effect with pressure has been observed for 2-napthol in a solvent mixture of ethanol, isopropanol, or acetone in ethane [53] and has been attributed to proximity to upper critical end point [53] as solubility is very sensitive to changes in pressure ($\partial y/\partial P$ is very large) near a critical end point.

4.3.1.3. Other effects

The competing effects of temperature on solvent density and solute vapor pressure lead to a crossover of solubility isotherms in SCFs. A distinct crossover point was observed in the cosolvent systems studied by Ekart et al. [53]. While a shift in crossover pressure was observed for a number of systems, Ting et al. [22] observed no significant shift in the crossover pressure of naproxen in the presence of cosolvents. Ethanol addition decreased the crossover pressure of sunflower oil from 40 MPa in pure CO_2 to 37.5 MPa and 35 MPa for 5 and 10 wt % ethanol, respectively [20]. The crossover pressure was also dependent on the cosolvent. For example, while the crossover pressure of o-HBA was higher than that of m-HBA in CO_2 + methanol, that of m-HBA was higher in CO_2 + acetone [91].

Increasing the hydrostatic pressure increases the melting point of a pure solid. However, when a solid is compressed in the presence of a supercritical fluid, the melting point of the solid decreases with increasing pressure due to the increasing solubility of the supercritical solvent in the heavy liquid phase. The addition of a cosolvent to a binary system can lower the melting temperature of the solid at a given pressure and change the T-P projection of the S-L-V melting curve [92]. Melting point depression was observed for 2-napthol in CO_2 + methanol and for naphthalene in CO_2 + n-pentane [92]. At a given pressure, the melting point of naphthalene decreased with increasing n-pentane concentration in CO_2. For a given cosolvent concentration, the melting point increased with increasing pressure. The larger melting point depression observed for the polar system (2-napthol + methanol) compared to the non-polar system (naphthalene + n-pentane) was attributed to the higher concentration of the cosolvent in the liquid phase due to H-bonding between solute and cosolvent [92].

4. 3.2. Effects of cosolvents on mass transfer

In addition to its effect on the solubility behavior of solutes, the addition of a cosolvent also affects the mass transfer properties such as diffusion coefficient. Smith et al. [93] studied the effect of methanol (5.5 mol %) on the diffusion of acridine and benzoic acid in $SCCO_2$ and reported that diffusion was greatly reduced by the presence of methanol and attributed this to the formation of solute-cosolvent clusters, size and mass of which increased due to chemical association of the solute with the cosolvent. Diffusivity of phenanthrene in $SCCO_2$ was little affected by methanol addition similar to its impact on solubility. However, little or no effect of secondary solvents [94], and an increase in diffusion coefficients with cosolvent addition have also been reported [95]. Karamat and Temelli [95] reported that ethanol addition resulted in the enhancement of diffusion coefficients of oleic acid and oleic acid methyl ester in $SCCO_2$.

In their study on the mass transport enhancement in cosolvent systems using SFE of a packed bed of β-naphthol-impregnated porous pellets with CO_2 + toluene, Abaroudi et al. [96] reported that the mass transfer coefficient and effective diffusivity increased with cosolvent concentration but to a lesser extent than solubility. Mass transfer modeling of $SCCO_2$ extraction of fungal lipids with ethanol [97, 98] and of sunflower oil [99] with different alcohol cosolvents (methanol, ethanol, butanol and hexanol) have also been investigated.

The enhancement in the extraction efficiency during analytical SFE achieved using modifiers has been attributed to the increase in solubility and modifier-matrix interactions, which lead to matrix swelling and displacement of analyte from sorption sites on the solid [100-102]. The modifier can cover active sites and prevent readsorption or partitioning of the analyte back onto the matrix active sites. Matrix swelling increases the exposure of interior volumes of the matrix to the solvent mixture enhancing the rate of mass transfer into solvent. Moisture in the sample can thus improve the accessibility of the analyte through matrix swelling. However, the presence of water can also interfere with the effectiveness of the extraction if it alters the sample matrix in such a way to hinder diffusion or if some analytes have more affinity to water than CO_2 [103].

4.3.3. Implications for fats and oils processing

Cosolvents can be used in the processing of fats and oils to improve the feasibility of a process by improving the solvent loading and/or selectivity. However, cosolvent addition brings additional considerations, such as increased complexity of the process design, which will determine the overall economics of the process.

4.3.3.1. Effect on solvent loading

Due to the increase in solvent loading or solubility enhancement obtained using a cosolvent, a required level of extraction yield can be obtained at a lower pressure. Such a pressure reduction makes extraction of solutes, which are sparingly soluble in $SCCO_2$ under practical conditions, such as phospholipids, possible.

The effects of ethanol addition on the extraction of oils such as sunflower [20, 99], cottonseed [104], corn germ [105] and pistachio [18] oils, vernonia oil [106], fungal lipids [97, 98] and cocoa butter [17, 107] have been investigated. Oil extraction yields were reported to increase with ethanol concentration. The rate of increase of extraction yield of pistachio oil was not constant such that the yield was more sensitive to changes in ethanol concentration in 0-5 wt% range and leveling off in the 5-10 wt % range [18]. Addition of ethanol has also been investigated as a means to improve extraction efficiency of carotene from carrots [108, 109] and lycopene and carotene from tomato paste waste [110]. Cosolvents can also be used in analytical applications [86], where unlike food applications, the cosolvents of choice are not restricted to ethanol.

$SCCO_2$ extraction of oilseeds yields an oil product with a very low phospholipid content [111] as phospholipids are only sparingly soluble in $SCCO_2$ at practical operating pressures [112, 113]. The low phospholipid content of $SCCO_2$ extracted oils is advantageous as it simplifies the subsequent refining process and decreases refining losses. However, a valuable by-product is left in the defatted flakes, which can be extracted using a cosolvent as the solubility of phospholipids can be improved significantly by the addition of a cosolvent such as ethanol [114]. Extraction of phospholipids from canola flakes [113, 115] and press cake [113] and soybean flakes [114, 116] was investigated using a two step procedure, involving an extraction step for oil removal followed by phospholipid extraction with ethanol addition into $SCCO_2$. The removal of phospholipids from canola meal [113] and the acetone-insoluble fraction obtained from crude canola lecithin (complex mixture of phospholipids, triglycerides and others such as glycerides, free fatty acids (FFA) and glycolipids) [115] was also investigated using CO_2 + ethanol. It should be noted that the extent of phospholipid extraction was dependent on the extraction pressure, type of cosolvent and cosolvent concentration used. For example, no significant level of phospholipids was extracted from defatted soybean flakes at 16.6 MPa using CO_2 + ethanol [116]. In their study on the deoiling of crude lecithin, Teberikler et al. [117] concluded that both acetone (10 wt %) and ethanol (5 wt %) were suitable cosolvents in terms of increasing the oil solubility without any considerable phospholipid extraction at 17 MPa where normally no oil extraction was possible.

Phospholipid extraction has also been observed during the $SCCO_2$ extraction of egg yolk lipids using alcohol cosolvents [112]. The extracted phospholipids were mainly concentrated in the later fractions and concentration in the extract increased with cosolvent concentration. The increase in the oil recovery achieved using ethanol from fish muscle [118] and dried fungi [98, 99] were partly due to an increase in the extraction of phospholipids. Boselli and Caboni [119] was able to extract phospholipids from dried egg yolk using an analytical-microscale SFE procedure without using cosolvents at 51.7 MPa and 313 K and attributed this to the high pressure used and the modification in the protein structure, leading to the destruction of lipid-protein aggregates due to the freezing thawing involved in the four cycle SFE procedure used, where freezing occurred due to the decompression step after each cycle.

4.3.3.2. Effect on selectivity

Selectivity of a separation process can be improved by the addition of cosolvents if there are specific interactions between the cosolvent and the solutes of interest as demonstrated for CO_2 + cosolvent systems containing a non-polar and a polar solute [37, 90, 120]. Lemert and Johnston [37] investigated the selectivities in cosolvent systems near critical end points. They reported that selectivity for naphthol in naphthol + phenanthrene + methanol + CO_2 system increased with methanol concentration due to H-bonding interactions between methanol and naphthol. Selectivity values increased by 50% with an increase in pressure from 15 to 35 MPa at higher methanol concentrations (6 mol %) at 308 K [37].

Different cosolvent effects observed for lipids can be exploited for fractionation of fats and oils, such as deacidification of oils. Brunner and Peter [21] reported an enhancement in the distribution factors of the FFA of palm oil by a factor of 2 by the addition of ethanol (at 344 K and 13.7 MPa) [21]. In continuous fractionation of palm oil [19], FFA content was reduced to less than 0.1% by the addition of ethanol (3.7-6.3 mol % at 17.1-24 MPa). However, a decrease in selectivity of the deacidification process with cosolvent addition has also been reported [121]. Turkay et al. [121] investigated the effect of static methanol addition (0.5 mL to 1 g seeds at 60 °C and 15 MPa) on the deacidification of black cumin seed oil. Significant enhancement of the extraction rate of both FA and neutral oil was observed especially in the first 15 min of the extraction when methanol was still in the extraction cell; however, methanol addition decreased the selectivity of the process such that the FFA content of the remaining oil was higher (7.8 versus 11.6%) [121].

Ibanez et al. [122] investigated the effect of ethanol addition on the fractional separation of tocopherols from olive pomace using two separators. While waxes, triglycerides and sterols were collected in the first separator, tocopherols were concentrated in the second separator. Ethanol was used to increase the polarity of CO_2, thus favoring the extraction of tocopherols; however, the effect of cosolvent addition on the selectivity of the process cannot be determined as no data were available for the case without ethanol addition.

Ethanol addition (5 wt % at 20 MPa and 333 K) improved the selectivity of pilot-scale column fractionation of orange roughy oil to remove peroxides and FA as the solubilities of FA and peroxides increased more quickly with ethanol content than those of wax esters and triglycerides [123]. The observed entrainer effect (an increase in both solubility and selectivity as defined in Section 4.3.1.1) is probably due to the specific interactions (H-bonding) between ethanol and FA and peroxides.

The selectivity will not be improved if there are no preferential interactions between the cosolvent and certain mixture components. Nilsson et al. [124] reported that ethanol addition decreased the selectivity of the fractionation of menhaden oil fatty acid ethyl esters (at 60 °C and 12.5 MPa with 5 wt % ethanol). In the fractionation of shark liver oil, the separation of squalene and tri- and diglycerides decreased with increasing ethanol concentration [123]. The separation factor between mono- and diglycerides in the quasi-ternary system, oleic glycerides + acetone + CO_2 decreased with increasing amount of cosolvent [125].

The decrease in solvent power resulting from the addition of cosolvents such as nitrogen and partitioning of the extract components between the cosolvent and extract-rich phase during solvent recovery can also be exploited to achieve fractionation (as discussed in Section 4.3.3.3). The use of binary gas mixtures (CO_2 + nitrogen) has been investigated for the selective extraction of pesticides from lipid containing matrices with minimal co-extraction of lipids [126].

4.3.3.3. Solvent recovery and other practical considerations

Despite all the advantages of cosolvent addition, this approach makes the process more complicated and overrides some of the major advantages associated with $SCCO_2$ processes. However, these aspects are usually not taken into consideration in various studies reporting the use of cosolvents.

A cosolvent can be introduced continuously into the system using a cosolvent pump or premixed cylinders. The difficulties associated with the use of premixed gas cylinders was summarized by Page et al. [30]. Continuous introduction of the solvent mixture using a cosolvent pump involves mixing the SCF and the compressed cosolvent prior to contact with the feed material. The use of a cosolvent pump however will increase the cost of the process. Alternatively, a cosolvent can be introduced by mixing it with the sample prior to extraction and allowing them to equilibrate. However, most of the cosolvent will then be removed from the extraction cell during dynamic extraction, resulting in a variable cosolvent concentration throughout the process.

One of the cited advantages of cosolvent addition, enhanced efficiency of solvent regeneration has been attributed to the effect of cosolvents on the temperature and pressure dependence of solubility, which enables the regeneration of the SCF to be achieved by a smaller change in pressure or by changing the temperature alone [21, 37, 90]. For example, at 13 MPa the solubility of palm oil decreased from about 2% to a negligible amount at 110 °C in CO_2 + ethanol mixtures, which made complete regeneration and recycling of the solvent by only a temperature change possible [21]. However, increasing the temperature to such high levels may not be feasible if the target solute is a heat labile biologically active minor lipid component.

The use of cosolvents will also lead to additional complexity in the solvent recovery steps [98, 107, 123, 127] as the cosolvent has to be separated from the extract, extraction residue and SCF. Additional separators would be needed and the separation would be more energy intensive as separation steps, such as distillation or evaporation would be needed to obtain the desired products and regenerate the solvent. The temperature and pressure of the separator need to be optimized to minimize loss of extract and coprecipitation of the cosolvent. Addition of atmospheric gases like N_2 and Ar to the CO_2 + extracts has been proposed as a means of solvent regeneration without changing the pressure and temperature [32]. However, this process would require an additional step for the separation of the atmospheric gases from CO_2.

Usually, the extract obtained after the separation of the SCF is a two-phase system, with a cosolvent-rich phase and an extract-rich phase where the extract components will be partitioned between these two phases to different extents. For example, the extracts obtained in the fractionation of shark liver oil consisted of a top ethanol phase, which was light colored and contained some odor compounds, and a squalene-rich, odorless and colorless bottom fraction [123]. In the extraction of cocoa butter and xanthines from cocoa nibs, most of the xanthines were dissolved in the ethanol fraction as the xanthines were not soluble in cocoa butter [107]. Another separation step involving a decanter or centrifuge will be needed to separate these phases.

The use of cosolvents can also complicate sample analysis as the cosolvent can degrade chromatographic performance or interfere with the detection of target analytes [128]. The use of helium-pressurized CO_2 cylinders may decrease the solvent power of CO_2 due to the presence of dissolved helium in CO_2. For example, solubility reductions in the range of 30-50 wt % were observed for soybean oil [129]. An increase in solvent loading may result in the co-extraction of undesirable compounds such as phospholipids, FFA and/or pigments (such as gossypol in cottonseed oil [104]) during the extraction of oils [20, 104]. Therefore, extraction parameters should be optimized to maximize the yield while minimizing extraction of undesirable compounds.

The effect of cosolvent addition on the sample matrix should also be considered. For example, cosolvent addition for oil extraction from oilseeds may alter the functional properties of the proteins in the meal. Ethanol addition improved the functional properties (foaming characteristics) of the extracted corn meals/protein isolates [105]. Arntfield et al. [130] concluded that the addition of cosolvents can result in significant protein denaturation of egg yolk proteins. Improved extraction of lipids from fish muscle has been attributed to the dissociation of phospholipid-protein complexes due to the increase in polarity resulting from ethanol addition [118]. Additional heat treatment required to remove cosolvent from the residue might lead to protein denaturation.

Study of the use of cosolvents for any process should therefore entail a case by case analysis, which takes into account the effect of the cosolvent not only on the solubility behavior but also on mass transfer properties, process design, quality of the extract and/or the residue, post processing steps such as compositional analysis and cost.

4.4. SUMMARY

Successful application of cosolvents in processing of fats and oils requires a good understanding of the effect of cosolvent addition on all aspects of processing such as solubility behavior, mass transfer, properties of extract and residue and overall economics of the process. Solubility enhancement achieved using a small amount of cosolvent results from an increase in solvent density and/or intermolecular interactions and can be used to improve the feasibility of a process by improving solvent loading and/or selectivity. Addition of a cosolvent improves the selectivity of a separation process if there are specific interactions between the cosolvent and one or more of the mixture components, such as H-bonding. Different cosolvent effects observed for lipids can be exploited for fractionation of fats and oils, such as deacidification of oils.

4.5. REFERENCES

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5. SOLUBILITY BEHAVIOR OF TERNARY SYSTEMS OF LIPIDS IN SUPERCRITICAL CARBON DIOXIDE[1]

5.1. INTRODUCTION

Supercritical fluid extraction [1], fractionation [2] and reactions [3] of fats and oils have been widely investigated in the last two decades. Successful application of supercritical fluid (SCF) technology to fats and oils processing requires a good understanding of the phase behavior of lipid components in supercritical carbon dioxide ($SCCO_2$). A systematic investigation of the binary systems of major and minor lipid components in $SCCO_2$ has been carried out to determine the general trends of binary solubility behavior as affected by operating conditions and solute properties (Chapters 2, 3, respectively). Study of binary systems provides valuable information on the fundamentals of solubility behavior that can be used as a basis for the study of multicomponent mixtures. However, information on multicomponent solubility behavior is essential as most processes involve multicomponent mixtures and solubility behavior in multicomponent mixtures deviate from the binary behavior. Although multicomponent solubility data have been reported for a number of lipid systems, a systematic investigation on multicomponent solubility behavior has not been carried out.

First part of the investigation on the solubility behavior of multicomponent mixtures involved the solubility behavior of ternary systems involving lipid + cosolvent + $SCCO_2$ to determine the effect of cosolvents on the solubility behavior of lipids and the implications for fats and oil processing (Chapter 4). In this study, a similar approach is extended to the ternary systems of $lipid_1 + lipid_2 + SCCO_2$ and higher systems containing three and four lipid components. Therefore, the objectives of this study were: a) to review and analyze the phase behavior of lipids in ternary and higher systems to determine the effect of operating parameters and feed composition on solubility behavior and separation efficiency, and b) to assess the implications of the findings for fractionation processes.

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[1] A version of this chapter will be submitted to the Journal of Supercritical Fluids for consideration for publication.

5.2. SOLUBILITY DATA AND ANALYSIS

Literature phase equilibrium data of ternary and higher (quaternary and quinary) systems of lipids and $SCCO_2$ have been compiled (Table 5.1). For systems where adequate number of data points were available, partition coefficients and selectivities were calculated and data were analyzed by plotting vapor phase concentration (solubility in $SCCO_2$), liquid phase concentration, partition coefficients and selectivities as a function of pressure to determine the effect of operating conditions and feed composition on solubility behavior. The ternary systems studied included two triglycerides (TG) (trilaurin (LLL)/tripalmitin (PPP), trimyristin (MMM)/PPP, LLL/MMM), two fatty acids (FA) (oleic acid (OA)/linoleic acid (LA)), two fatty acid methyl esters (FAME) (methyl myristate (MeM)/methyl palmitate (MeP) and methyl oleate (MeO)/methyl linoleate (MeL)), FA/TG (OA/triolein (OOO)), and FAME/FA (MeO/OA) in $SCCO_2$. The quaternary and quinary systems analyzed contained three TG (LLL/MMM/PPP), three glycerides (monoolein (MO)/diolein (DO)/OOO) and four TG (PPP/palmitoyl-dioleoylglycerol (POO)/oleoyl-dipalmitoylglycerol (PPO)/OOO), respectively, in $SCCO_2$.

Binary solubility data were also included in the analysis to determine any deviation from binary behavior. Binary data from the same researchers were used to

Table 5.1. Literature phase equilibrium data of ternary and higher (quaternary and quinary) systems of lipids and $SCCO_2$.

System	T (K)	P (MPa)	Composition	Ref.	Binary ref.
Ternary systems[a]					
Triglycerides					
(1) LLL/PPP	313	9.1-24.7	-	4[d]	4[d]
(2) MMM/PPP	313	9.2-30.4			
(3) LLL/MMM	313	9.2-24.9			
(4) OOO/ SSS	313	20	50/50[b]	5[e]	5[f]
Fatty acids					
(5) OA/LA	313, 333	7.3-28.7	15/85, 50/50, 75/25[c]	6[g]	7[h]
(6) SA/PA	313	20	50/50[b]	5[i]	5[j]
Fatty acid methyl esters					
(7) MeO/MeL	313,333	7.30-21	35/65, 50/50, 65/35[c]	6	7
(8) MeM/MeP	313,323	8-13	12.5/87.5, 25/75, 37.5/62.5, 50/50, 62.5/37.5, 75/25, 87.5/12.5[c]	8	8
Fatty acid ester/Fatty acid					
(9) OA/MeO	313,333	9.2-26.2	[illegible]/75, 50/50, 75/25[c]	9	10
Fatty acid/Triglyceride					
(10) OA/OOO	313-333	20-30	80/20, 50/50, 30/70[b]	11	11
Quaternary systems					
Triglycerides					
(11) LLL/MMM/PPP	313	9.2-25	-	4	4
Glycerides					
(12) MO/DO/OOO	333	17.2-30.9	10/10/80, 33/33/33[b]	12	12
Quinary system					
Triglycerides					
(13) PPP/OOO/PPO/POO	313, 333	17.2-31	9.1/29.8/16.2/43.4[b]	13	13

[a] LLL: trilaurin, PPP: tripalmitin, MMM: trimyristin, OOO: triolein, SSS: tristearin, OA: oleic acid, LA: linoleic acid, SA: stearic acid, PA: palmitic acid, MeO: methyl oleate, MeL: methyl lineolate, MO: monoolein, DO: diolein, POO: palmitoyl-dioleoylglycerol, PPO: oleoyl-dipalmitoylglycerol

[b] wt %, [c] mol %

[d] uncertainty of the measurement (standard error) = 6 %, [e] ± 0.1 w/w*10^2, [f] ± 0.04 w/w*10^2, [g] reproducibility within ± 0.5 mol %, experimental uncertainties <2% of measured value or 0.2 mol% whichever is greater, [h] average standard deviation (reproducibility) = ± 0.005 in mole fraction with maximum deviation between duplicates of ± 0.02, accuracy estimated to be ± 5% of reported value, [i] ± 1.0 w/w*10^2, j' ± 0.3 w/w*10^2

minimize variation between data. Solubility ratios, defined as the ratio of solubility in the multicomponent system to binary solubility under the same operating conditions were calculated to determine the effect of the presence of another solute on solubility behavior. Solubility ratios of >1 indicate solubility enhancement, whereas solubility diminution results in values <1. When solubility values under the same operating conditions were not available, values measured at pressures within ±0.5 MPa were used in the calculation of solubility ratio. For TG systems, ratios of partition coefficients (ratio of partition coefficient in the multicomponent system to binary partition coefficient under the same operating conditions) were also calculated.

Partition coefficients are used as correlating variables for systems where vapor phase concentrations are dependent on feed concentration. Partition coefficient is by definition the ratio of concentration of the solute in the vapor phase to that in the liquid phase. Any concentration unit (such as mole fraction or weight fraction) can be used for the calculation; however, consistent units should be used for both concentrations and for different components within a system. Concentrations in the liquid phase have been expressed either on a solvent-inclusive [6-11] or on a solvent-free basis [4, 12, 13]. When the liquid phase cannot be sampled and analyzed the solute concentration in the liquid phase is determined from mass balance calculations on a solvent-free basis as in the case of TG mixtures [4, 12, 13]. The partition coefficients calculated in this manner will reflect the degree of enrichment in the respective phases (such that values <1 and >1 indicate enrichment in the liquid and vapor phases, respectively) and would be systematically lower than solvent-inclusive values. They may also be qualitatively different from solvent-inclusive values as demonstrated by Stoldt et al. [14] for soybean oil deodorizer distillate components. This deviation should be considered while comparing partition coefficient trends from different studies. Solvent-free partition coefficients in binary systems can be taken as equal to binary solubility values (as the solute concentration (as mole or weight fraction) in the liquid phase is approximately one due to low solute solubility in $SCCO_2$).

In order to investigate the effect of operating conditions and feed composition on the efficiency of separation, selectivity values were calculated as the ratio of the partition coefficients of solutes of interest. For solid mixtures, selectivity is determined as the ratio of the solubilities of the solutes of interest. Selectivities predicted by the binary data were also calculated for comparison purposes. Selectivity values calculated on solvent-inclusive and solvent-free basis will be equal. When data under the same operating conditions were not available, values measured at pressures within ±0.5 MPa were used in the calculation of binary selectivities.

5.3. RESULTS AND DISCUSSION

5.3.1. Phase behavior in solid lipid systems

5.3.1.1. Solubility behavior

In solid mixtures, the solubility of each solid is independent of the solid mixture composition such that the concentration of components in the solid phase will have no effect on the solubility of individual components. Fluid phase solubilities become dependent on the composition of the condensed phase when the solute melts or when it is liquid [4, 15, 16] or when solid solutions are used [17]. In S-L-V mixtures, the vapor phase concentration will be independent of feed concentration as long as the same solid is in excess [15]. For TG mixtures LLL/PPP and MMM/PPP, solubilities of mixture components were not affected when the relative amounts of TG charged to the cell were changed by 20% [4].

Solubility enhancement has been well documented in ternary systems containing two solid components and $SCCO_2$ [15, 18]. Lucien and Foster [15] provided an extensive review of the solubility behavior of ternary solid mixtures in $SCCO_2$. In ternary solid mixtures, generally the solubility of the less soluble component is enhanced over the binary value, whereas that of the other component is enhanced to a lesser degree or is not affected. Solubility diminution for both or one of the solutes has also been reported [15, 18]. Observed solubility enhancement has been attributed to the upper critical end point (UCEP) of the ternary systems being lower than that of binary systems and to the cosolvent effect. At a certain temperature and pressure, the ternary system is closer to its UCEP resulting in higher solubilities, since solubility increases as UCEP is approached [18]. The difference in the enhancement of mixture components (the fact that the solubility of one solid may be enhanced to a greater extent than the other in the same system) however cannot be explained by the approach to the mixture UCEP.

Solubility enhancement in such systems can be explained by the "cosolvent effect". Addition of a cosolvent improves the solubility of a solute due to an increase in solvent density and/or intermolecular interactions between the cosolvent and solute as discussed in detail in Chapter 4. In ternary systems with two solid solutes, the more soluble component behaves as a cosolvent and enhances the solubility of the other compound. This cosolvent effect has been supported by the observation that the solubility enhancement increases with the fluid phase concentration of the more soluble component [19, 20]. Therefore, a more soluble solid causes a more significant increase in the solubility of the less soluble component. The level of solubility enhancement obtained in solid systems is typically much lower than that observed in cosolvent systems due to the relatively low concentration of the more soluble component in solid systems compared to concentrations used in cosolvent systems [20].

Solubility ratios of lipid components calculated for the investigated lipid systems have been summarized in Table 5.2. In TG systems, the highest value (2.59) was

Table 5.2. Solubility ratio (ratio of ternary solubility to solubility in the binary system under the same operating conditions) of lipids in studied systems.

	Solubility Ratio		P (MPa)	T (K)
System 1	**LLL**	**PPP**		
	0.84-0.98	0.89-2.59	9.1-24.7	313
System 2	**MMM**	**PPP**		
	0.41-0.99	1.35-1.79	9.2-30.4	313
System 3	**LLL**	**MMM**		
	0.56-1.41	0.15-1.15	9.2-24.9	313
System 5	**OA**	**LA**		
OA/LA mol%				
15/85	0.23	0.66-0.83	15.9/9.5-26.3	313
50/50	0.26-0.36	0.30-0.33	16.4-28.0/10.3-26.9	313
75/25	0.46-0.48	0.17-0.14	16.2-21.0/10.2-13.7	313
15/85	0.32	0.35	13.27	333
50/50	0.38	0.28-0.54	28.7/10.3-22.3	333
75/25	0.61	-	23.4	333
System 7	**MeO**	**MeL**		
MeO/MeL mol%				
35/65	0.29	0.59	9.3/9.3	313
50/50	0.25-0.28	0.29-0.33	9.2-10.9/9.2-10.9	313
65/35	0.53	-	7.5-9.7	313
35/65	0.20-0.29	-	11.4-14.9	333
50/50	-	0.38-0.62	12.1-20	333
65/35	0.40-0.47	-	11.8-14.8	333
System 8	**MeM**	**MeP**		
MeM/MeP mol%				
87.5/12.5	0.68-0.51	0.44-0.17	8-9	313
75/25	0.44-0.81	0.99-0.69	8-9	313
62.5/37.5	1.05	4.59	8	313
50/50	0.16-0.26	0.82-0.53	8-9	313
37.5/62.5	0.19-0.18	1.99-0.87	8-9	313
25/75	0.25-0.07	3.21-0.46	8-9/8-10	313
12.5/87.5	0.25-0.04	2.70-0.38	8-9/8-10	313
87.5/12.5	1.82-0.93	0.23	8-11	323
75/25	0.20-0.59	0.09-0.17	8-11	323
62.5/37.5	1.70-0.60	0.35-0.61	8-11	323
50/50	0.42/0.77	0.29/0.62	8-11/8-12	323
37.5/62.5	0.29-0.39	0.44-1.08	8-11/8-12	323
25/75	0.13/0.62	0.32-1.33	8-11/8-13	323
12.5/87.5	0.08-0.48	0.44-1.12	8-11/8-13	323

Table 5.2. continued

	Solubility Ratio			Pressure (MPa)	Temperature (K)
System 9	**MeO**		**OA**		
MeO/OA mol%					
50/50	0.42		0.75	10.01	313
25/75	0.2-0.65		0.75	11.2-13.3/13.3	333
50/50	0.41		0.50	14.1	333
75/25	0.29		1	13.9	333
System 10	**OA**		**OOO**		
OA/OOO wt.%					
80/ 20	0.87-1.42		-	20-30	313
50/50	0.48-7.43		0.40-0.44	20-30/25-30	313
30/70	0.30-1.96		0.44-0.81	20-30	313
80/20	0.88-1.48		-	20-30	333
50/50	0.49-1.35		0.55	20-30/25	333
30/70	0.35-0.55		0.36	20-30/25	333
System 11	**LLL**	**MMM**	**PPP**		
LLL/MMM /PPP	0.48-1.30	0.32-1.20	1.52-2.37	9.2-25	313

observed for PPP in LLL/PPP mixture whereas in cosolvent systems solubility ratios as high as 60 were obtained (Chapter 4). In the cosolvent lipid systems, cosolvent concentrations as high as 12 mol % were used whereas the solubility of TG were in the range of 10^{-3}-10^{-8} mole fraction under the investigated conditions.

In S-L-V systems however the observed trends could not be explained solely by the cosolvent effect [15]. When a eutectic solid mixture undergoes melting, one solute (minor solute) will undergo complete melting and thus be present only in the liquid phase, whereas the other solute (excess solute) will undergo partial melting and thus be present in both solid and liquid phases. In ternary S-L-V systems, solubility enhancement was observed for the solute present as excess solid, whereas the solubility of the minor solute was either enhanced slightly or depressed. This behavior has been attributed to the vapor pressure effect. Partial melting of the excess solute increases its vapor pressure, which results in the observed solubility enhancement. Similarly, solubility diminution observed for the minor solute can be attributed to a decrease in its vapor pressure due to the presence of the excess solid in the liquid phase. However, it should be noted that this vapor pressure effect (leading to solubility diminution) was mainly observed for systems, which show L-V equilibria in binary systems [15].

Intermolecular interactions (between the solutes in ternary systems and between the solute and cosolvent in cosolvent systems) are the major contributors to the cosolvent effect. In cosolvent systems, the largest solubility enhancements were achieved when there were specific interactions between the cosolvent and the solute (such as H-bonding interactions between ethanol and FA) (Chapter 4). Similarly, in ternary systems containing polar solutes, specific solute-solute interactions resulted in large solubility enhancements whereas for systems in which solute-solute interactions mainly involved dispersion forces (systems containing nonpolar-nonpolar and nonpolar-polar pairs), solubility enhancement barely exceeded 20% (when the solubility of the more soluble component, acting as a cosolvent was in the range of 10^{-4}-10^{-3} mole fraction) [14].

Solubility behavior in systems containing solute mixtures and a cosolvent is determined by both solute-cosolvent and solute-solute interactions. Saquing et al. [21] investigated the solubility of a solid mixture of m-hydroxybenzoic acid (HBA) + o-HBA in a CO_2 + cosolvent (3.5 mol% methanol) mixture to determine the net effect of solute-solute and solute-cosolvent interactions on the solubility of polar solutes. Although the solubility of m-HBA was enhanced considerably by o-HBA in the ternary system, no further enhancement was observed in the quaternary (cosolvent) systems compared to ternary cosolvent system, which indicated that solute-solute interactions had no significant influence on solubility in the quaternary system. These findings and the large cosolvent effects observed suggest that each solute in the quaternary system interacted preferentially with the cosolvent through H-bonding interactions such that the functional groups of the solutes were engaged and there were no sites for specific solute-solute interactions. The cosolvent can also affect the solubility of one of the mixture components indirectly by raising the concentration of the other component [20].

5.3.1.2. Melting behavior

Melting behavior in a ternary system of two solids in $SCCO_2$ differs from that in binary systems of each solid with $SCCO_2$ and of the two solids. Similar to binary systems [22], melting point depression has been reported in various ternary systems of solids in $SCCO_2$ [15, 23-25]. The addition of a cosolvent to a binary system can also lower the melting temperature of the solid and change the T-P projection of the S-L-V melting curve [26].

Solubility trends observed in ternary/multicomponent systems of solids and $SCCO_2$ can be categorized based on the phase equilibria existing in the system [15]. Therefore, the melting behavior of ternary/multicomponent mixtures should be determined to facilitate accurate interpretation of ternary data. Melting behavior of ternary and quaternary TG systems has not been studied. The only information comes from the observation of Bamberger et al. [4] at a single temperature and pressure. They observed that LLL/MMM and LLL/MMM/PPP mixtures melted whereas PPP/MMM and LLL/PPP mixtures were solid at 313 K and 15 MPa. Although this information is useful, it is limited as it does not apply to a wide experimental range. Furthermore, for those systems, which were observed to melt at 15 MPa, it is not possible to ascertain if these systems exhibited S-L-V or L-V equilibria under the experimental conditions.

5.3.1.3. Solubility behavior of triglyceride systems

Solubility ratios for TG (in systems 1-3 and 11 in Table 5.1) were in the range of 0.15-2.59 corresponding to 85% diminution to 159% enhancement (Table 5.2). Solubility of TG under the investigated conditions was between 10^{-3}-10^{-8} mole fraction and increased with decreasing carbon number. TG data were analyzed both by component and by mixture to establish general trends of solubility behavior as affected by mixture composition and operating conditions. As pointed out above, the phase equilibria present in TG mixtures are unconfirmed. A comparison of solubility behavior observed in unconfirmed systems with those of confirmed equilibria can be used to determine the phase equilibria present. Using such an approach, Lucien and Foster [15] classified LLL/MMM systems as exhibiting L-V equlibria, whereas PPP/MMM and LLL/PPP systems were classified as unconfirmed S-L-V equilibria.

In the LLL/PPP system (Fig. 5.1), LLL showed slight solubility diminution whereas PPP solubility was enhanced by up to 159% and this enhancement increased linearly with pressure. Similarly, in the MMM/PPP system (Fig. 5.2), the lower molecular weight MMM showed solubility diminution (0.41-0.99, 59-1%), which decreased with pressure, whereas the higher molecular weight component, PPP solubility was enhanced (1.35-1.79; 35-79%).

In the LLL/MMM mixture (Fig. 5.3), solubility ratio of LLL was in the range of 0.56-0.65, which corresponded to a solubility diminution by 35-44% in the pressure range 11-24.8 MPa, whereas at 9.3 MPa, solubility ratio was 1.41 corresponding to a

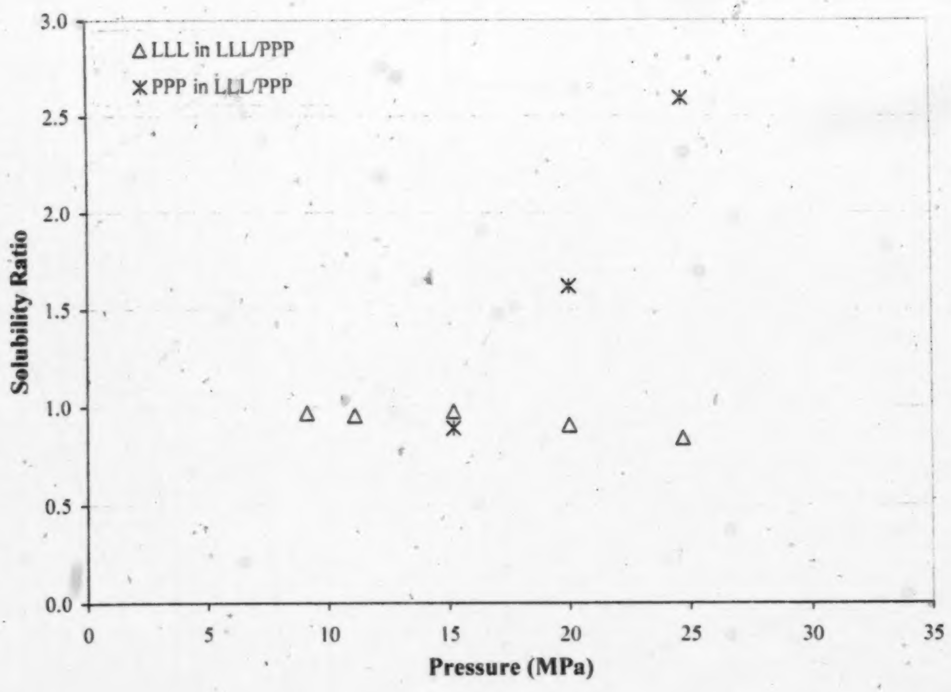


Figure 5.1. Solubility ratio of LLL and PPP in ternary system LLL/PPP/CO_2 at 313 K (Data from Ref. 4).

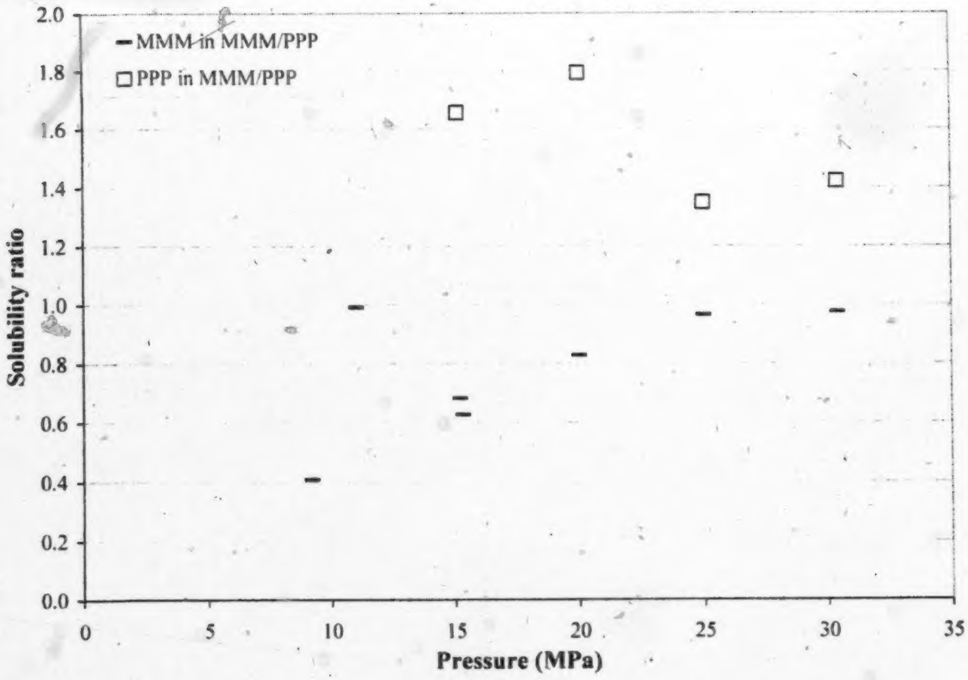


Figure 5.2. Solubility ratio of MMM and PPP in ternary system MMM/PPP/CO_2 at 313 K (Data from Ref. 4).

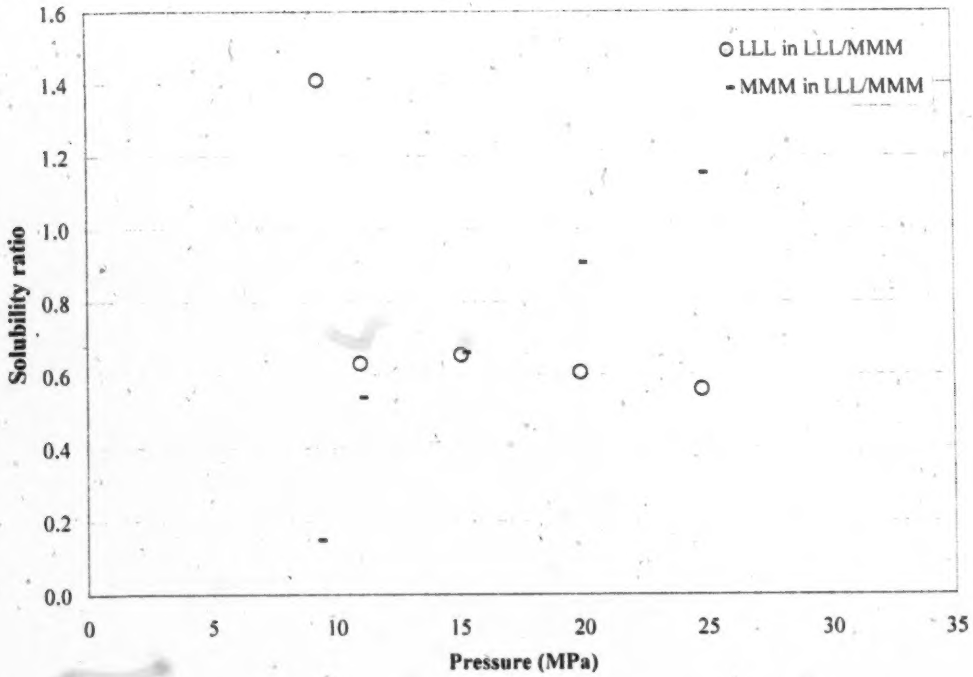


Figure 5.3. Solubility ratio of LLL and MMM in ternary system LLL/MMM/CO_2 at 313 K (Data from Ref. 4).

41% enhancement. The solubility ratio of MMM increased with pressure from 0.15 at 9.3 MPa to 1.15 at 24.8 MPa. It should be noted that for this system different conclusions could be drawn using the partition coefficient approach. The partition coefficient of LLL in the ternary system was similar to that in the binary system whereas that of MMM was enhanced in the ternary system as shown in Figure 5.4.

In the quaternary mixture, LLL/MMM/PPP in $SCCO_2$ (Fig. 5.5), solubility diminution was observed for LLL in the pressure range 11-25 MPa by 35-52%, whereas at 9 MPa, LLL solubility was enhanced by 30%. Solubility ratio of MMM increased with pressure from 0.32 at 9.2 MPa to 1.2 at 25 MPa. The solubility of the least soluble solute, PPP, was enhanced in the pressure range of 15-25 MPa by 52-137%. Partition coefficients however indicated enhancement over the entire range (except at the lowest pressure studied, 9 MPa) for all solutes (Fig. 5.4).

Data were also analyzed by mixture to compare the solubility behavior of the specific TG in different systems. Solubility diminution was observed for LLL in all mixtures (Fig. 5.6). While the diminution was similar in ternary LLL/MMM and quaternary LLL/MMM/PPP mixtures, it was substantially smaller in the LLL/PPP (3-16%) mixture. Solubility enhancement was observed for LLL in ternary LLL/MMM and quaternary LLL/MMM/PPP mixtures only at the lowest pressure investigated (9 MPa). Partition coefficients of LLL in the binary, ternary (LLL/MMM) and quaternary (LLL/MMM/PPP) systems (Fig. 5.4) were similar. Solubility ratio of MMM was in the range of 0.15-1.20 and increased with pressure in all the systems investigated (Fig. 5.7). In the MMM/PPP mixture the ratio approached 1 with pressure, whereas solubility enhancement was observed for LLL/MMM, LLL/MMM/PPP at the highest pressure

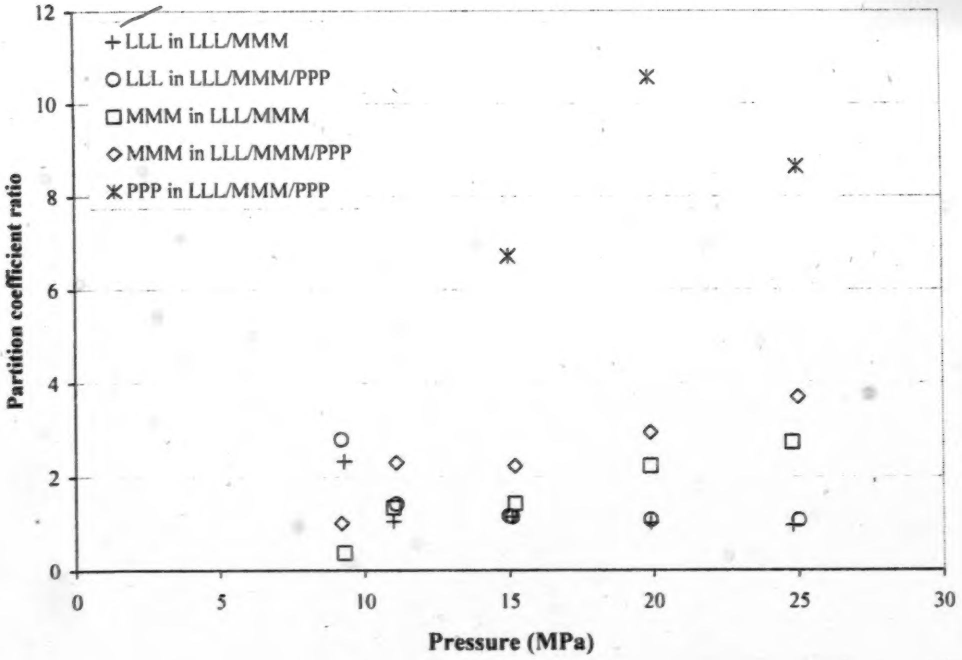


Figure 5.4. Ratio of partition coefficients of LLL, MMM and PPP in ternary and quaternary systems at 313 K (Data from Ref. 4).

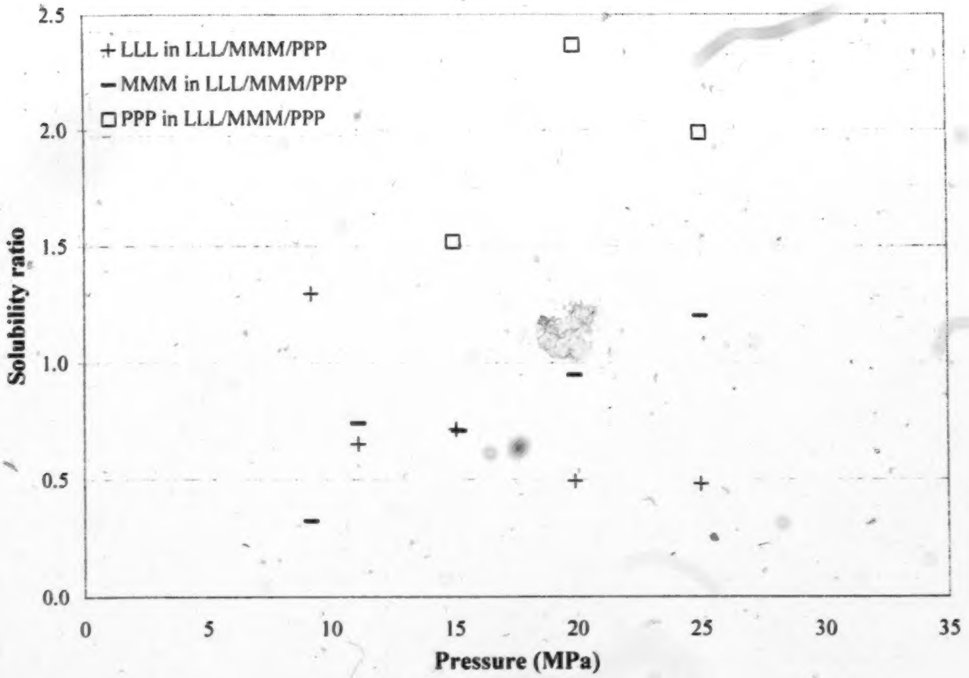


Figure 5.5. Solubility ratio of LLL, MMM and PPP in quaternary system LLL/MMM/PPP/CO_2 at 313 K (Data from Ref. 4).

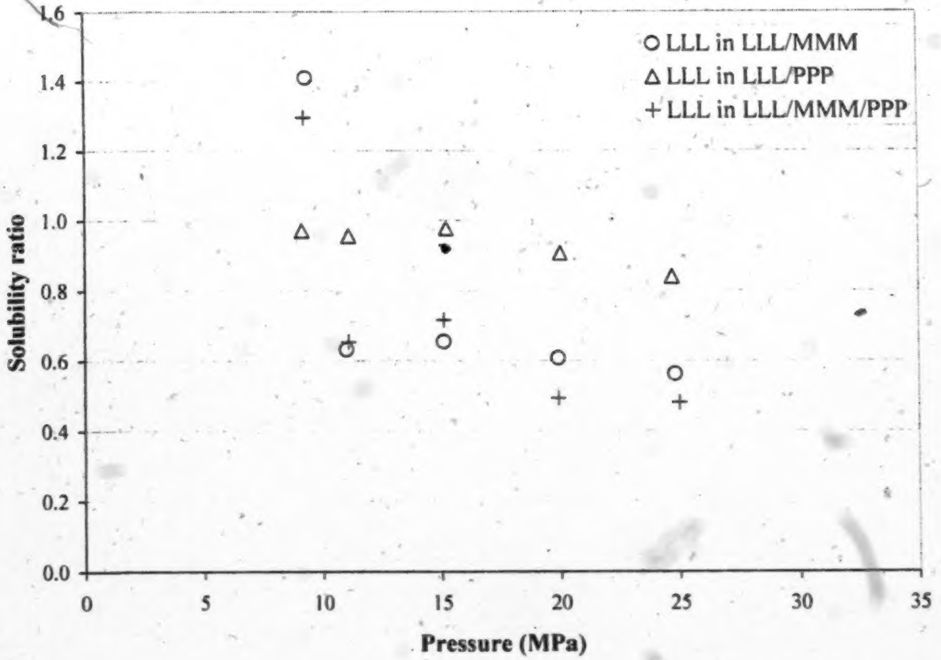


Figure 5.6. Solubility ratio of LLL in ternary and quaternary mixtures (LLL/MMM, LLL/PPP, LLL/MMM/PPP) of triglycerides and $SCCO_2$ at 313 K (Data from Ref. 4).

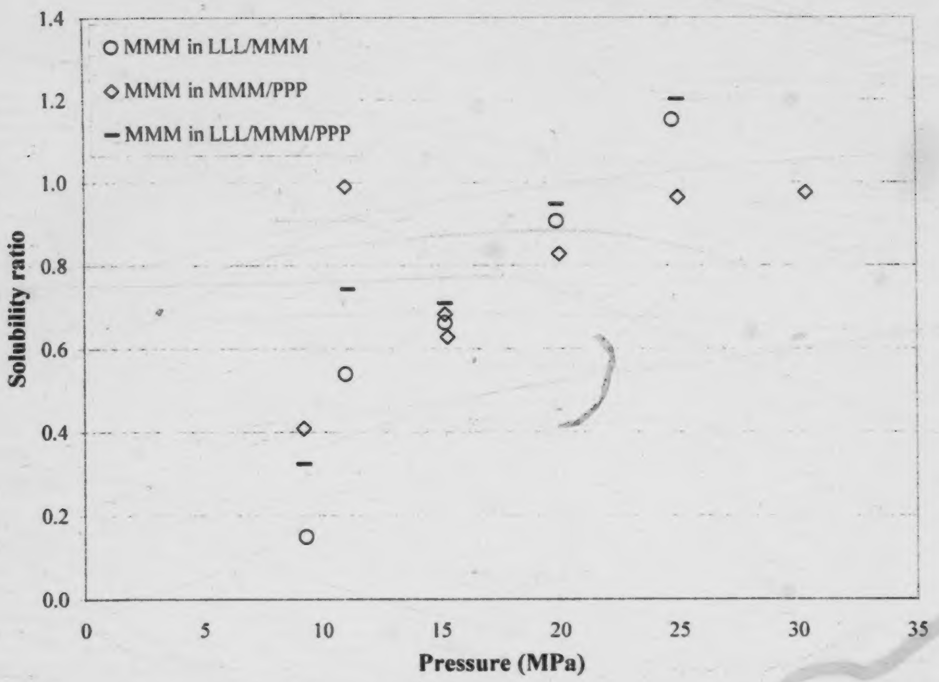


Figure 5.7. Solubility ratio of MMM in ternary and quaternary mixtures (LLL/MMM, MMM/PPP, LLL/MMM/PPP) of triglycerides and $SCCO_2$ at 313 K (Data from Ref. 4).

investigated (25 MPa). The partition coefficient of MMM was enhanced in LLL/MMM and LLL/MMM/PPP mixtures (Fig. 5.4). PPP solubility was enhanced in all mixtures (Fig. 5.8). Partition coefficient of PPP in the quaternary system was higher than that in the binary system (Fig. 5.4).

Nilsson and Hudson [13] studied a quinary mixture containing four TG (OOO/POO/PPO/PPP) in $SCCO_2$ (using a reaction mixture with 1.5% by-products) in the pressure range 17.2-31 MPa at 313 and 333 K. Binary solubility data of OOO and PPP were reported at 313 and 333 K; however, no binary data were available for mixed TG. Only partition coefficients of mixture components were reported for the quinary mixture. Binary OOO solubility was higher than that of solid PPP at 313 K, whereas at 333 K, PPP solubility was higher as it melted at this temperature.

The partition coefficient of OOO was similar to its binary value at 313 K (Figs. 5.9, 5.10), whereas that of PPP was enhanced substantially (Figs. 5.11, 5.12). At 333 K, the ratios decreased with pressure from 1.83 to 1 for OOO and from 1.89 to 1 for PPP (Figs. 5.10, 5.12). The significant enhancement observed for PPP at 313 K can be attributed to melting of PPP in the quinary mixture.

At 313 K, partition coefficient of OOO was the lowest while the order of that for other TG was dependent on pressure. For example, PPO was the highest except at the highest pressure studied where PPP was the highest (Fig. 5.13). At 333 K, the partition coefficients were in the order of PPP>PPO>POO>OOO at all pressures studied (Fig. 5.14). Partition coefficients of PPO, POO and OOO decreased with temperature (in the temperature range 313-333 K and in the pressure range 17.2-31 MPa) and there appeared to be a crossover of isotherms at the higher pressures (31 MPa) (Figs. 5.9, 5.15). For PPP

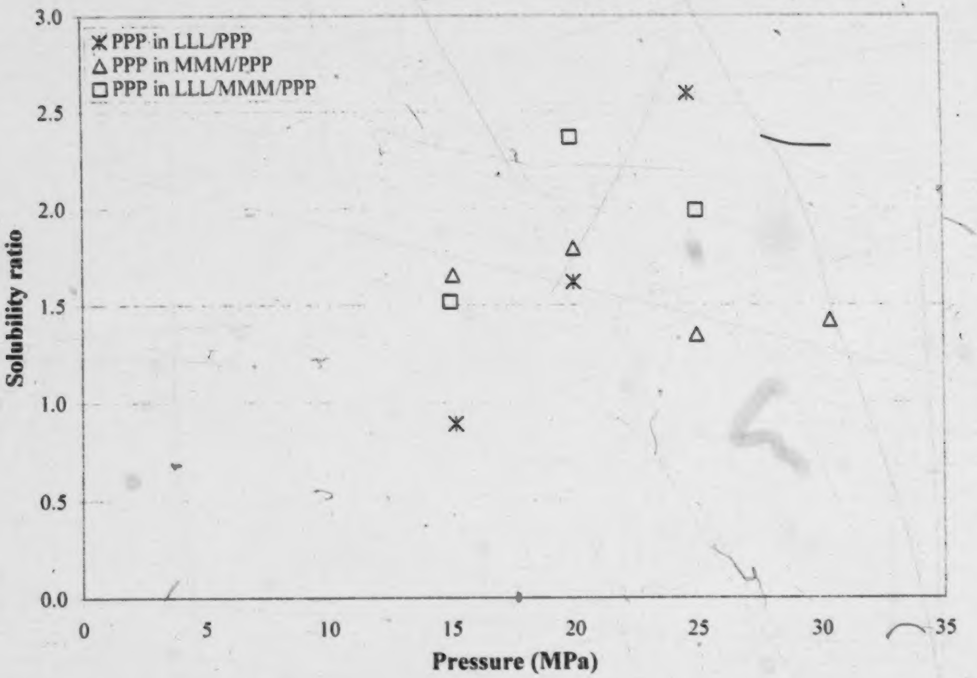


Figure 5.8. Solubility ratio of PPP in ternary and quaternary mixtures (LLL, PPP, MMM/PPP, LLL/MMM/PPP) of triglycerides and $SCCO_2$ at 313 K (Data from Ref. 4).

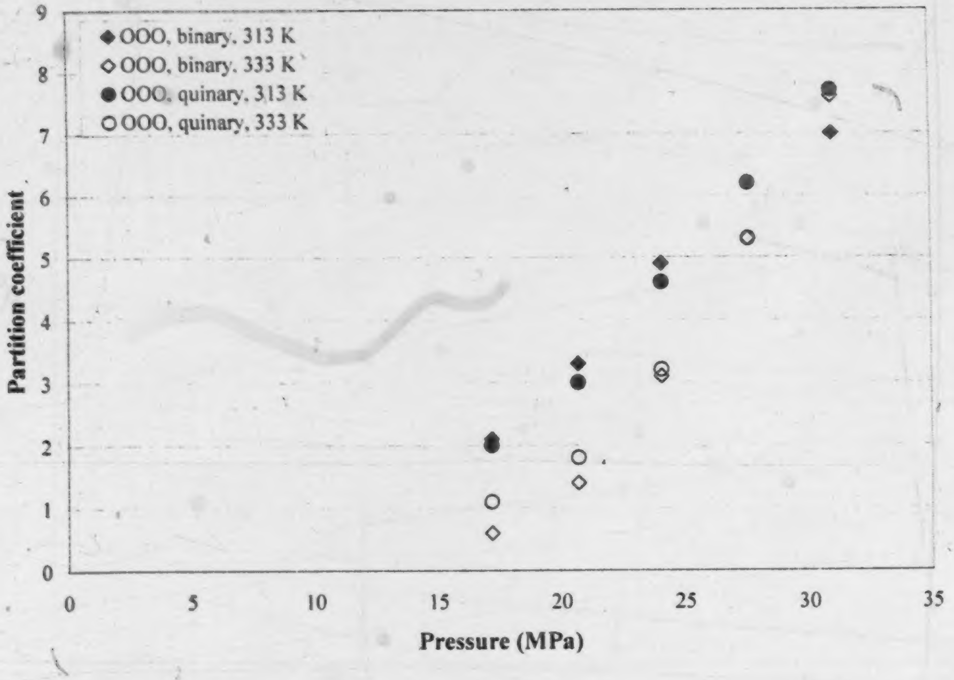


Figure 5.9. Binary and quinary (PPP/OOO/POO/PPO) partition coefficient isotherms of OOO in $SCCO_2$ (Data from Ref. 13).

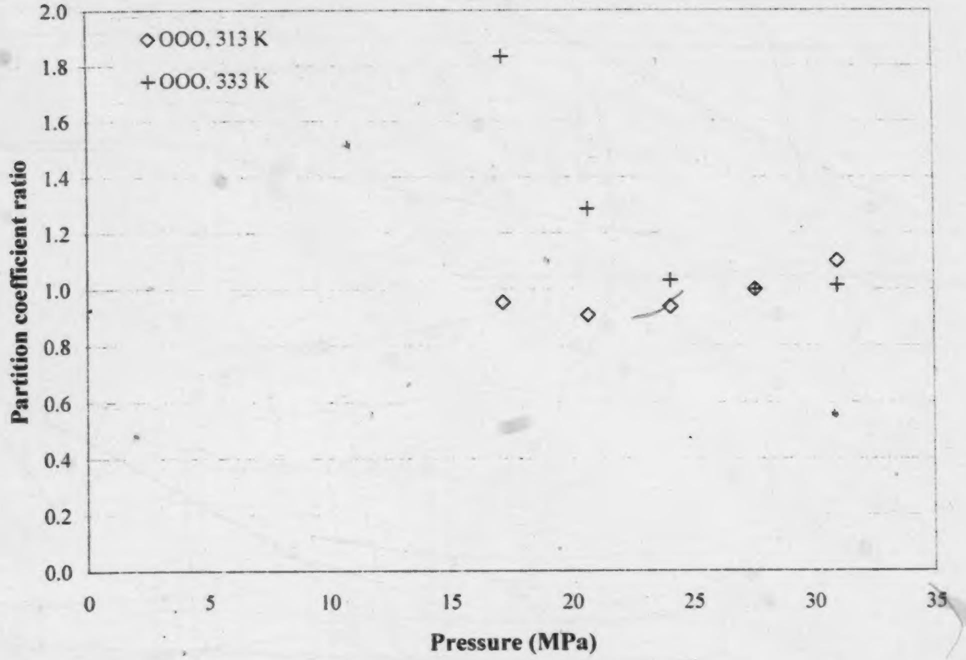


Figure 5.10. Ratio of partition coefficients of OOO in the quinary system PPP/OOO/POO/PPO/CO_2 (Data from Ref. 13).

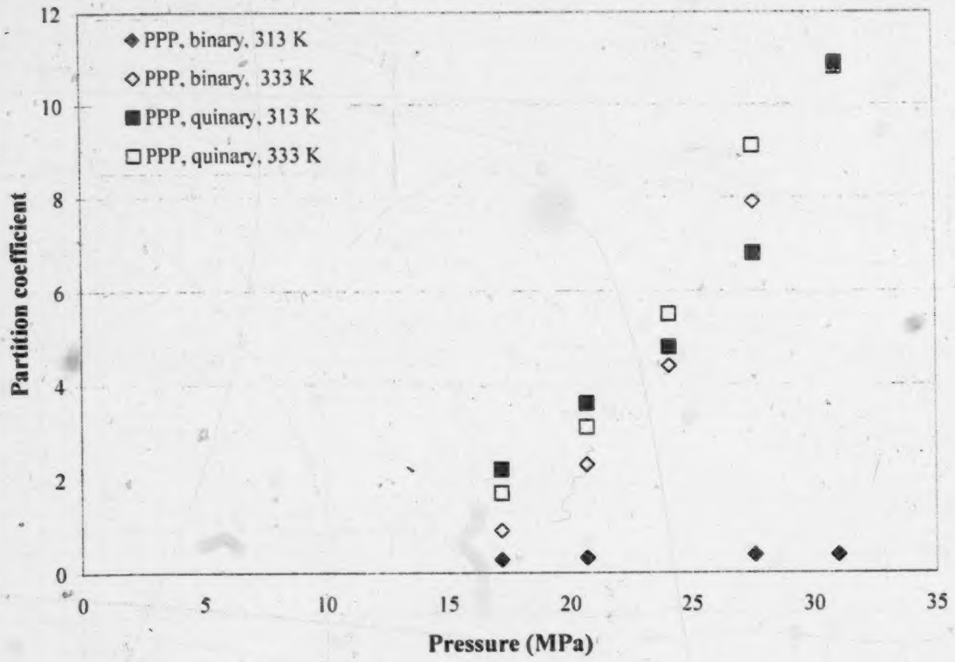


Figure 5.11. Binary and quinary (PPP/OOO/POO/PPO) partition coefficient isotherms of PPP in $SCCO_2$ (Data from Ref. 13).

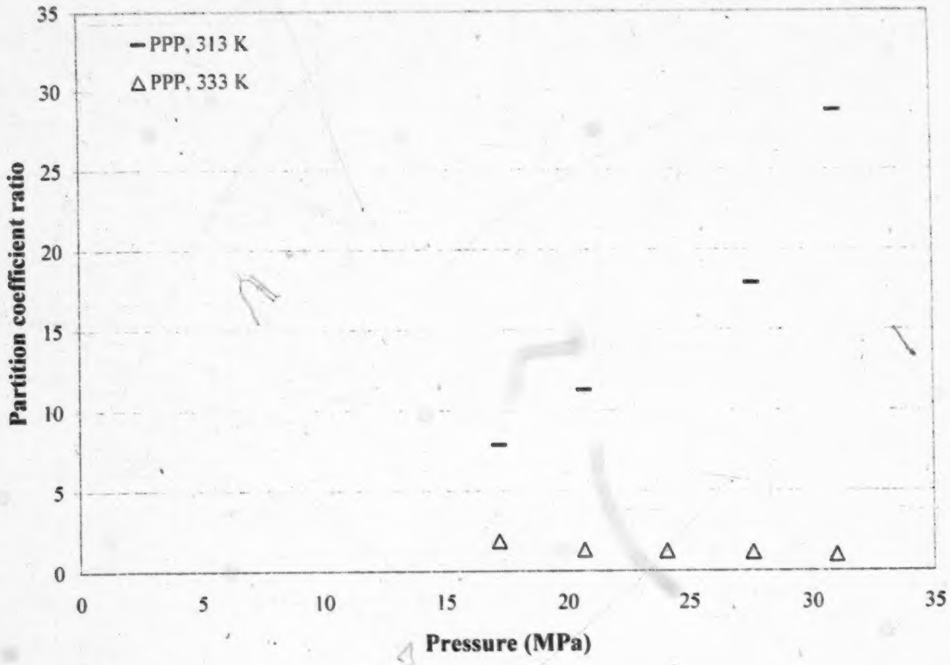


Figure 5.12. Ratio of partition coefficients of PPP in the quinary system PPP/OOO/POO/PPO/CO_2 (Data from Ref. 13).

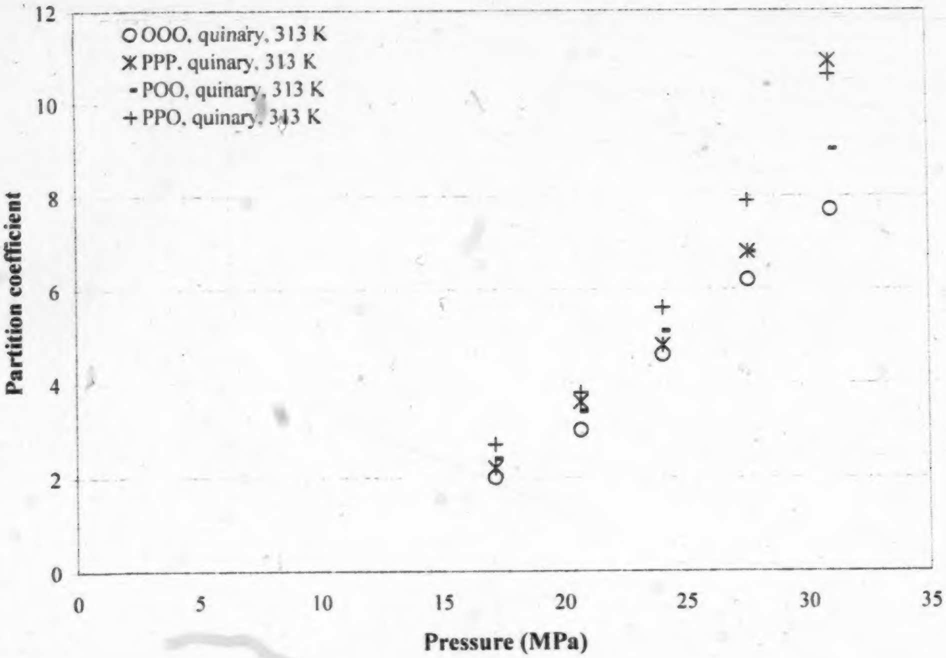


Figure 5.13. Quinary (PPP/OOO/POO/PPO) partition coefficients of PPP, OOO, POO, PPO in $SCCO_2$ at 313 K (Data from Ref. 13).

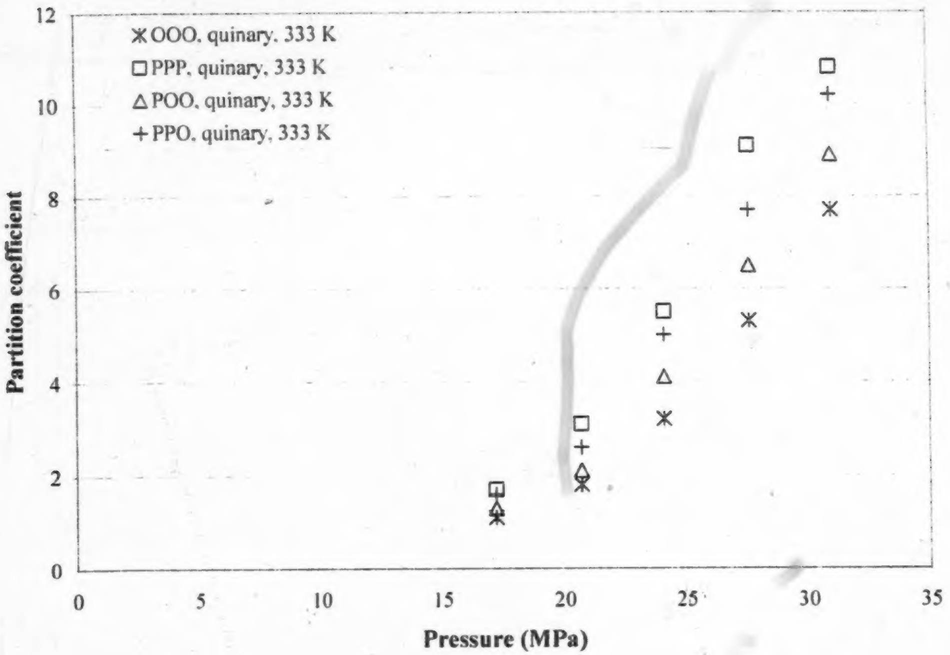


Figure 5.14. Quinary (PPP/OOO/POO/PPO) partition coefficients of PPP, OOO, POO, PPO in $SCCO_2$ at 333 K (Data from Ref. 13).

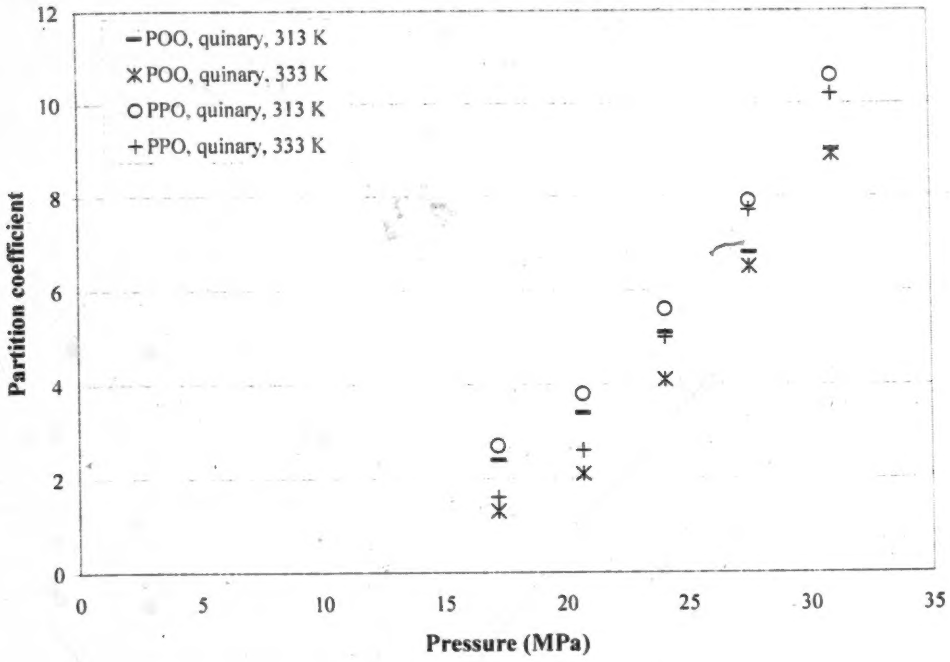


Figure 5.15. Quinary (PPP/OOO/POO/PPO) partition coefficients of POO and PPO in $SCCO_2$ (Data from Ref. 13).

however this crossover took place at a lower pressure, such that the solubility increased with temperature at pressures>21 MPa (but solubilities were similar at the highest pressure) (Fig. 5.11). The temperature and pressure range investigated should be noted while reporting the temperature dependence of partition coefficients. The difference in the temperature dependence of the partition coefficient values of mixture components may have important implications for fractionation processes as discussed in Section 5.3.3. The solubility behavior of the TG mixtures discussed above was affected by existing phase equilibria (solubility and physical state/melting behavior of the studied solutes) and pressure. Solubility diminution for both solutes as observed in LLL/MMM mixtures has generally been observed for liquid mixtures (L-V) and will be discussed in Section 5.3.2. The trends observed in LLL/PPP, MMM/PPP, and LLL/MMM/PPP are consistent with those observed in ternary S-L-V mixtures. The solubility enhancement observed in these systems can also be partly attributed to the cosolvent effect of the more soluble component. This cosolvent effect depends on its concentration/solubility in the supercritical phase, which in turn increases with pressure. The pressure dependence of solubility ratio can be explained by the effect of pressure on the solubility of individual mixture components. The higher solubility enhancement of PPP compared to MMM in the presence of LLL can be attributed to the higher solubility of LLL in this system.

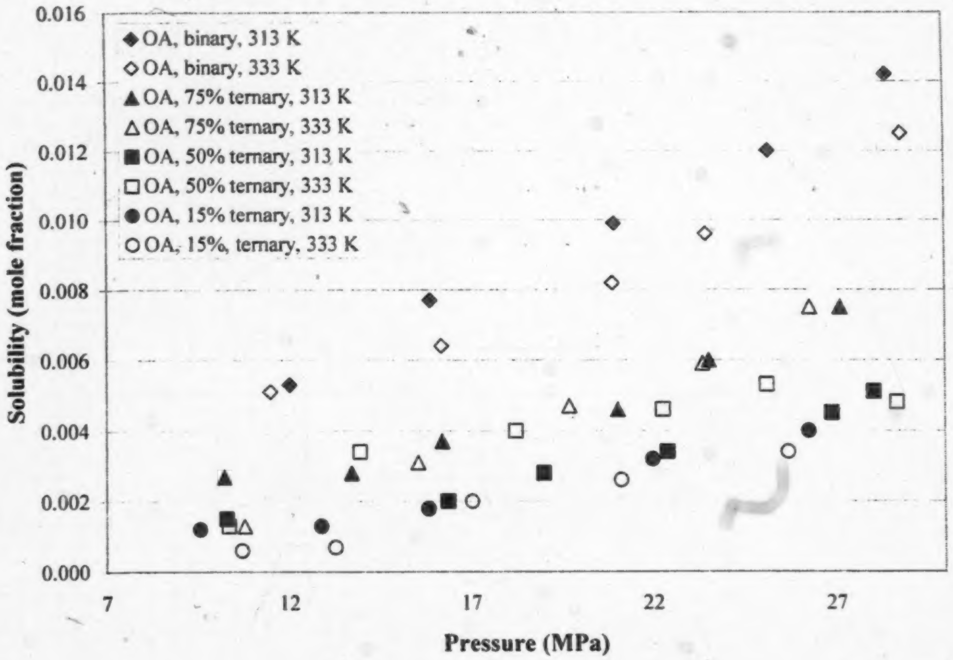
The investigation of the effect of the presence of other solutes on the solubility behavior provides valuable insight into the solubility behavior of multicomponent mixtures; however, the partition coefficient of the solutes will determine the extent of separation achievable in the system. As demonstrated earlier, the trends observed for partition coefficients can be quantitatively and at times qualitatively different from those observed for solubility. The deviant results observed at lowest pressures can be due to the higher error involved in the solubility measurements at conditions closer to the critical point where the effect of operating conditions on solubility is maximal. Information on melting behavior and phase equilibria existing in multicomponent systems is pivotal in the study of the solubility behavior of these systems as trends observed can be classified according to the phase equilibria present.

5.3.2. Phase behavior in liquid systems

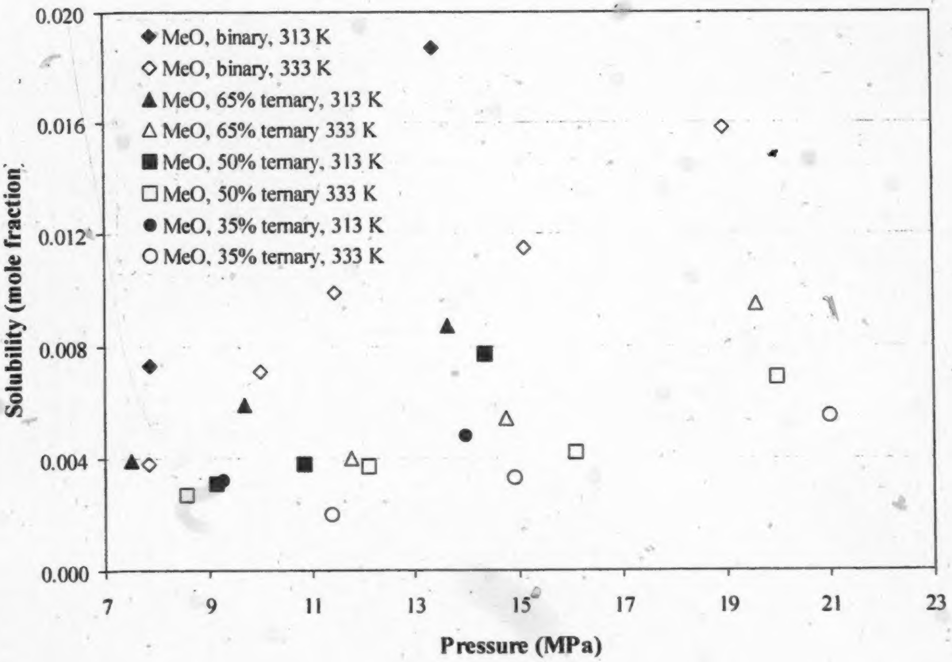
Solubility ratios calculated for liquid mixtures have been presented in Table 5.2. Calculated ratios were in the range of 0.14-0.83 for system 5, 0.20-0.62 for system 7, 0.07-4.59 for system 8, 0.2-1 for system 9 and 0.30-7.43 for system 10. However, it should be noted that solubility ratio could not be calculated for every data point as binary data at similar pressures were not available. Vapor phase concentration versus pressure plots provide a complete picture of the deviation of ternary solubilities from binary values.

5.3.2.1. Vapor phase composition

The ternary systems 5 (OA/LA) and 7 (MeO/MeL) displayed solubility diminution for both solutes in all the investigated mixtures over the entire experimental range. For these systems, vapor phase concentration increased with feed concentration (Figs. 5.16, 5.17, Table 5.2) such that the extent of the diminution was dependent on solute feed concentration. The effect of concentration on solubility ratio can be clearly seen for LA in the OA + LA mixture at 313 K (Table 5.2). Solubility diminution for LA decreased from 83-86% to 17-34% with concentration in the feed (from 25 to 85 mole %



5.16. Binary and ternary (OA/LA) solubility isotherms of OA in $SCCO_2$ (Data from Refs. 6 and 7).



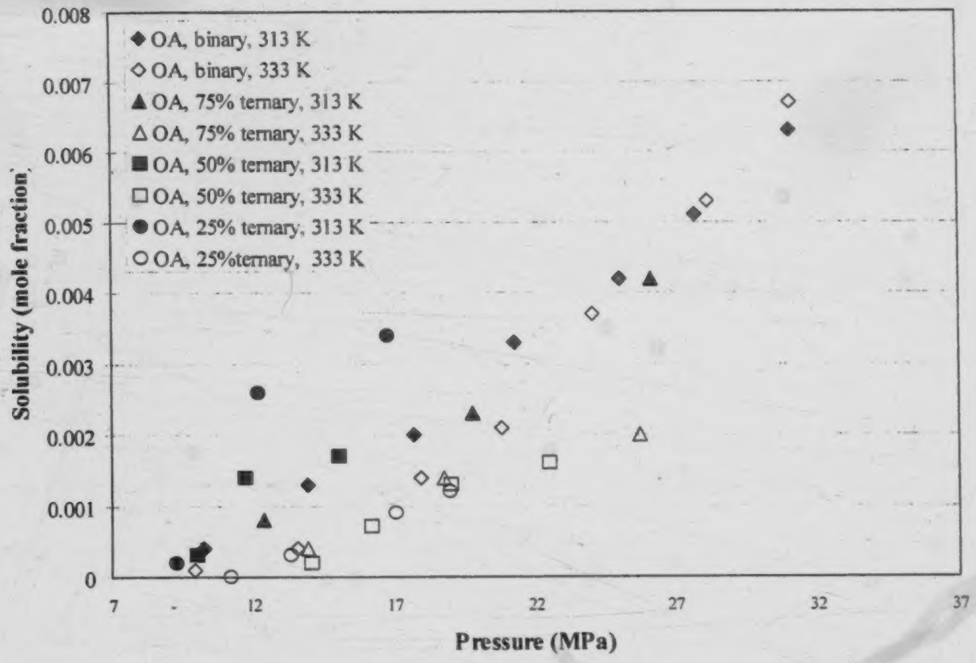
5.17. Binary and ternary (MeO/MeL) solubility isotherms of MeO in $SCCO_2$ (Data from Refs. 6 and 7).

LA), while ternary vapor phase concentration for OA was highest in the 75% OA mixture and solubilities in 25 and 50% OA mixtures were similar (Fig. 5.16).

For systems 8 (MeM/MeP), 9 (OA/MeO) and 10 (OA/OOO), while the general trend was solubility diminution for both solutes, solubility enhancement was also observed for one of the solutes in certain mixtures (Table 2, Fig. 5.18). Solubility enhancement was observed in MeM/MeP fatty acid ester mixture (Table 5.2) and for OA in the OA/MeO (Fig. 5.18) and OA/OOO mixtures (Table 5.2). In the ester system, solubility enhancements as high as 359% and 82% were observed for MeP (in 37.5% MeP mixture at 8 MPa and 313 K) and MeM (in 87.5% MeM mixture at 8 MPa and 323 K), respectively. In the OA/MeO system, solubility diminution was observed for MeO in all mixtures under the experimental conditions, whereas solubility of OA was enhanced in the 25% OA mixture at 313 K (Fig. 5.18, Table 5.2). Solubility of OA appeared to be independent of concentration in other mixtures at this temperature, whereas solubility diminution was observed for 50 and 75% OA mixtures at 333 K (Fig. 5.18). Solubility of OA was also enhanced in the FA/TG mixture (Table 5.2). In the OA/OOO system, no trend could be established in solubility behavior with pressure; furthermore, the reported data were not consistent with the figure provided in the original paper. The analysis of this system was included; however, no attempt was made to draw any conclusions.

5.3.2.2. Liquid phase composition

At any given condition, concentration of a component in the liquid phase is a function of initial feed composition, CO_2 solubility in the liquid phase and solute solubility in the vapor phase. Liquid phase concentrations of the different components



5.18. Binary and ternary (OA/MeO) solubility isotherms of OA in $SCCO_2$ (Data from Refs. 9 and 10).

studied decreased with increasing pressure and decreasing feed concentration but to different extents (Figs. 5.19-5.23).

Pressure dependence of liquid phase concentrations arises from pressure dependence of solute solubility in $SCCO_2$ and CO_2 solubility in the liquid phase. Solute concentration in the liquid phase decreased with pressure due to increasing CO_2 concentration in the liquid phase (Figs. 5.24-5.27) and increasing solute solubility in the vapor phase.

Liquid phase concentration will deviate from the initial feed concentration due to solute solubility in $SCCO_2$ and CO_2 solubility in the liquid phase. The net effect of a change in initial feed composition on liquid phase concentration will be determined by the relative effect of feed concentration on each of these factors. CO_2 solubility in the liquid phase was dependent on feed composition for the investigated systems. It increased with increasing LA/decreasing OA concentration (Fig.5.24) and with increasing MeO/decreasing OA concentration (Fig. 5.25). In the MeO/MeL system, CO_2 solubility was independent of feed composition (Fig. 5.26), whereas it appeared to increase with MeM concentration in the MeM/MeP system (Fig. 5.27).

Liquid phase concentrations of solutes increased with feed concentration for both solutes in systems 5, 7, 8 and 9 (Figs. 5.19-5.23); however, the effect of feed concentration on liquid phase MeO and MeL concentrations in system 7 (Fig. 5.20) and MeO concentration in system 9 (Fig. 5.23) was smaller than that observed for other solutes. Concentration effect was dependent on pressure such that it decreased with pressure for both solutes in systems 7 and 8 and for MeO in system 9 (Figs. 5.20, 5.21, 5.23).

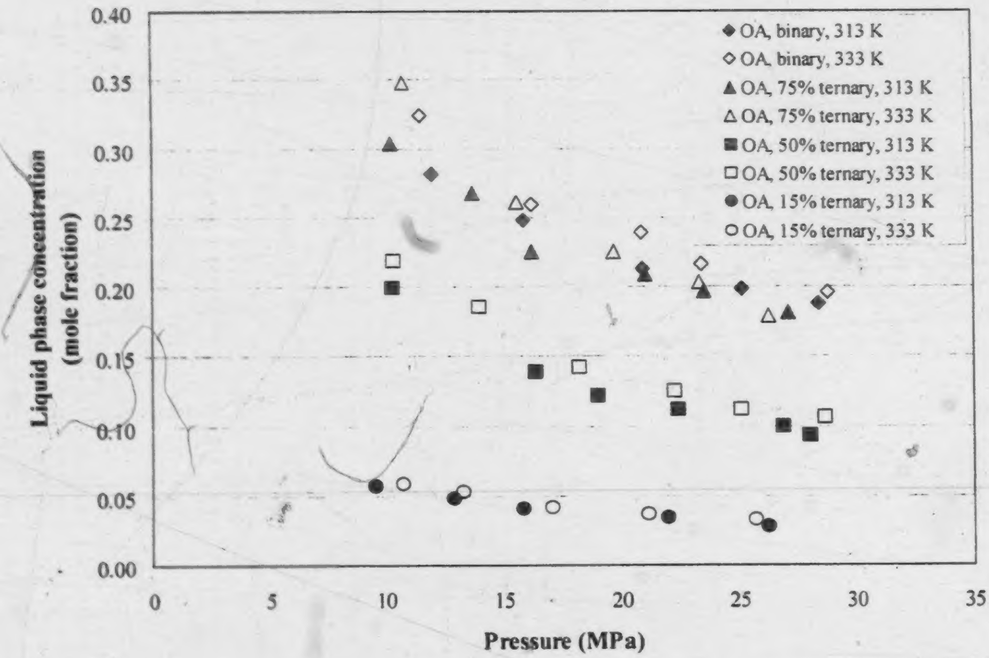


Figure 5.19. Liquid phase concentration of OA in binary and ternary mixtures of OA/LA and CO_2 (Data from Refs. 6 and 7).

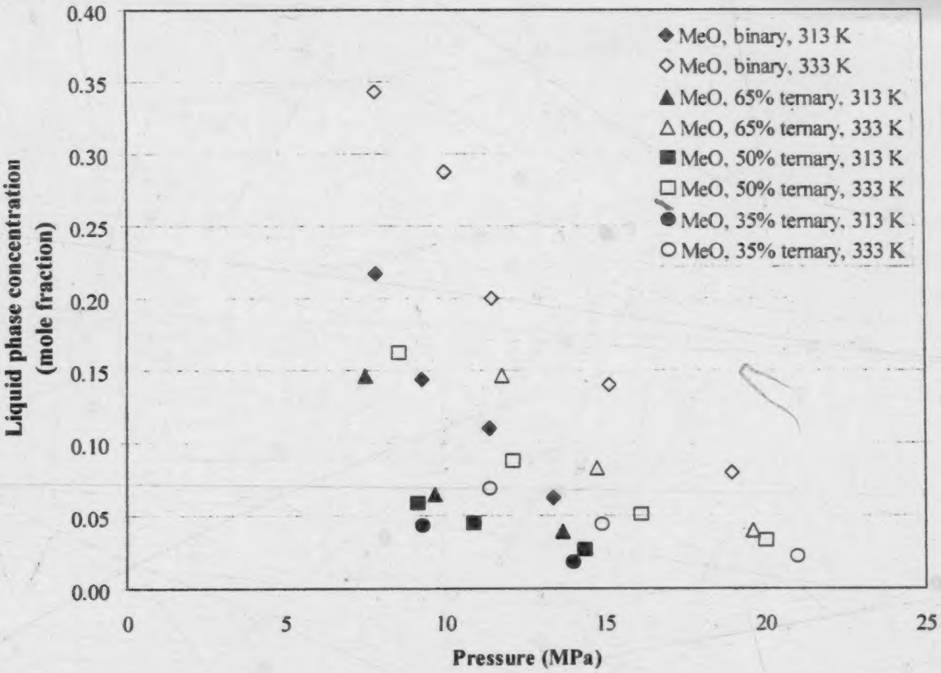


Figure 5.20. Liquid phase concentration of MeO in binary and ternary mixtures of MeO/MeL and CO_2 (Data from Refs. 6 and 7).

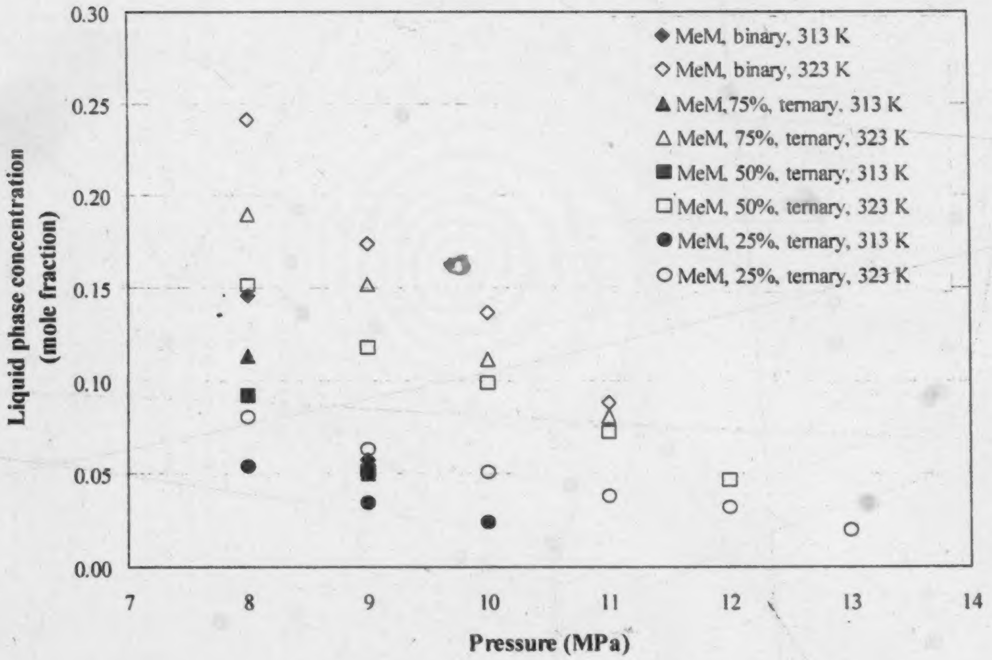


Figure 5.21. Liquid phase concentration of MeM in binary and ternary mixtures of MeM/MeP and CO_2 (Data from Ref. 8).

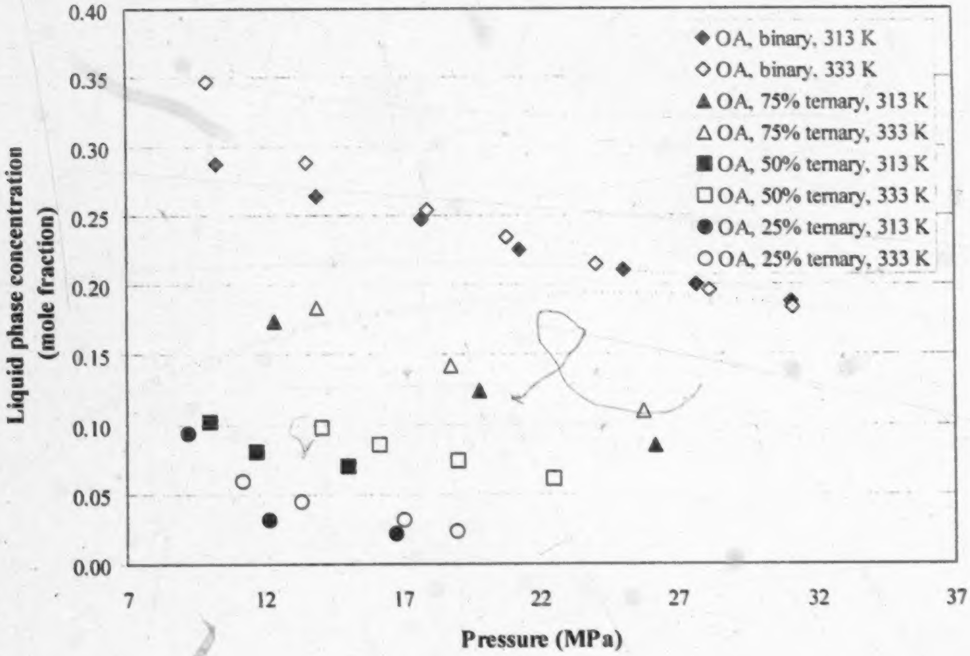


Figure 5.22. Liquid phase concentration of OA in binary and ternary mixtures of OA/MeO and CO_2 (Data from Refs. 9 and 10).

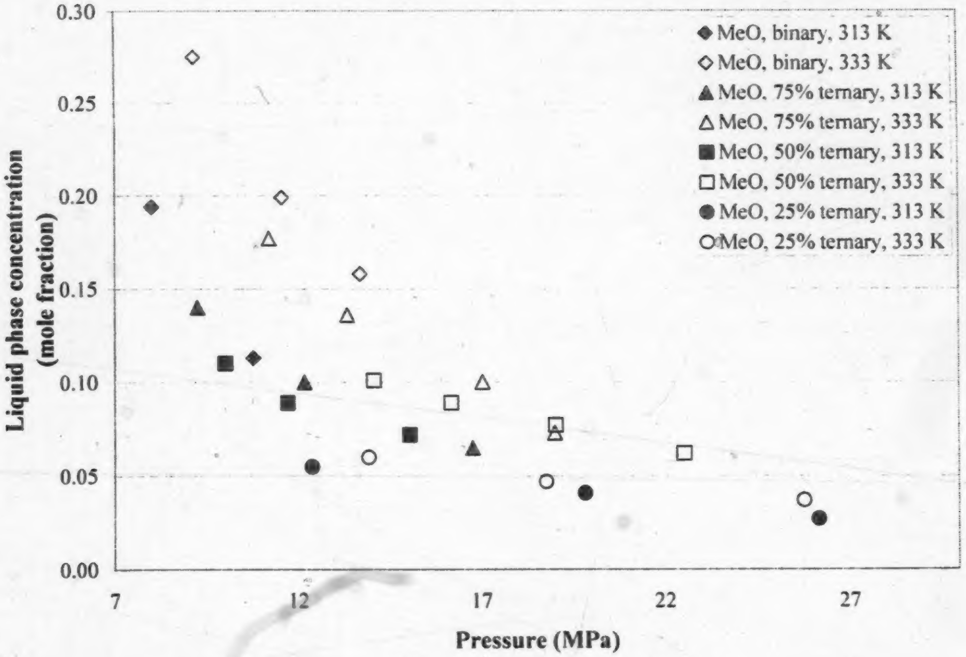


Figure 5.23. Liquid phase concentration of MeO in binary and ternary mixtures of OA/MeO and CO_2 (Data from Refs. 9 and 10).

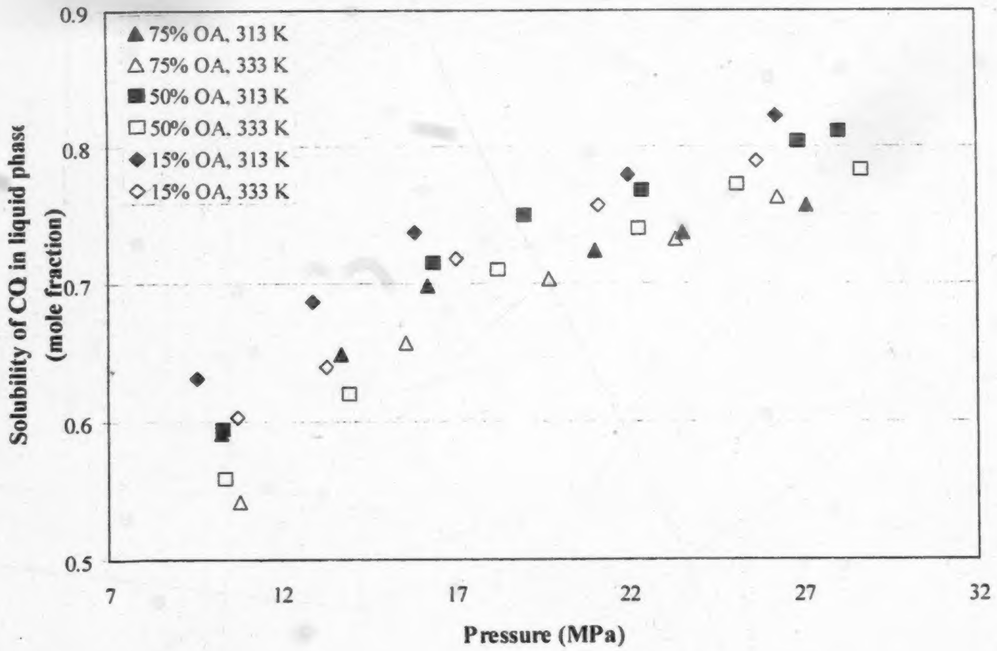


Figure 5.24. Solubility isotherms of CO_2 in OA/LA mixture (Data from Ref. 6).

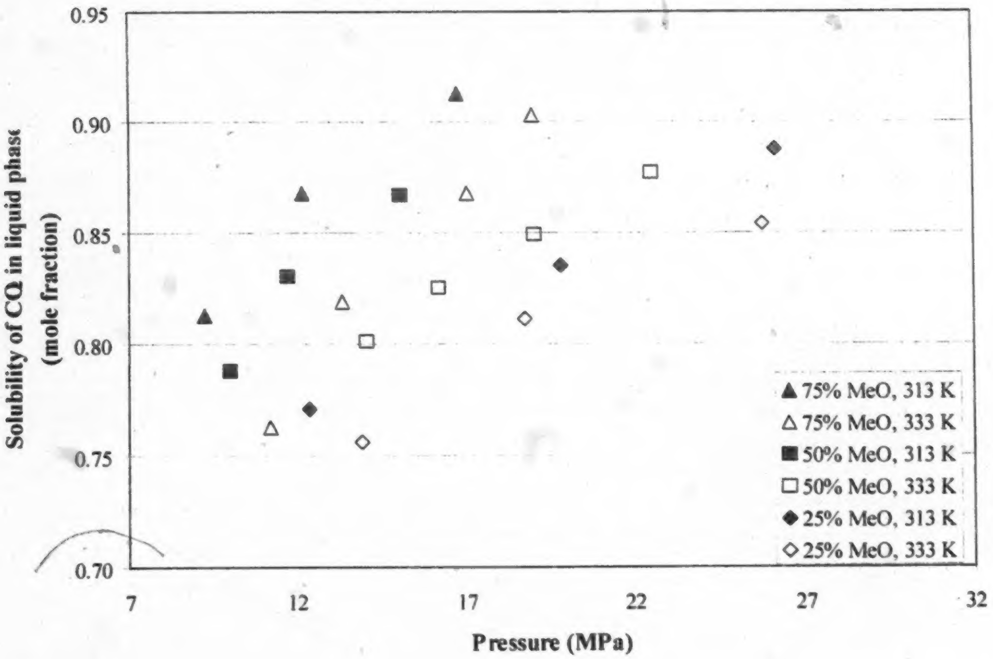


Figure 5.25. Solubility isotherms of CO_2 in MeO/OA mixture (Data from Ref. 9).

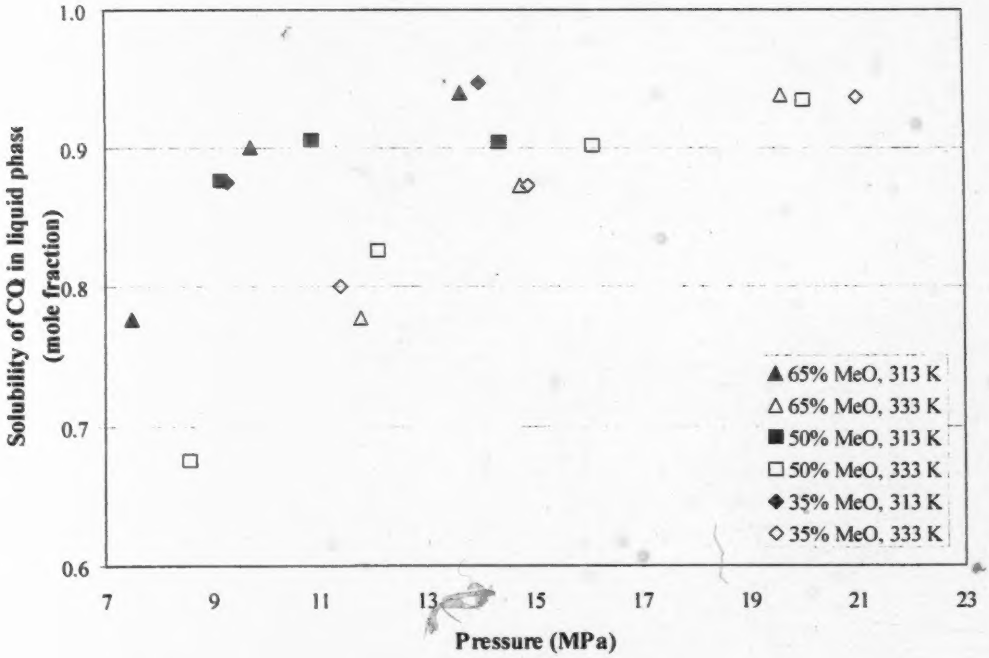


Figure 5.26. Solubility isotherms of CO_2 in MeO/MeL mixture (Data from Ref. 6).

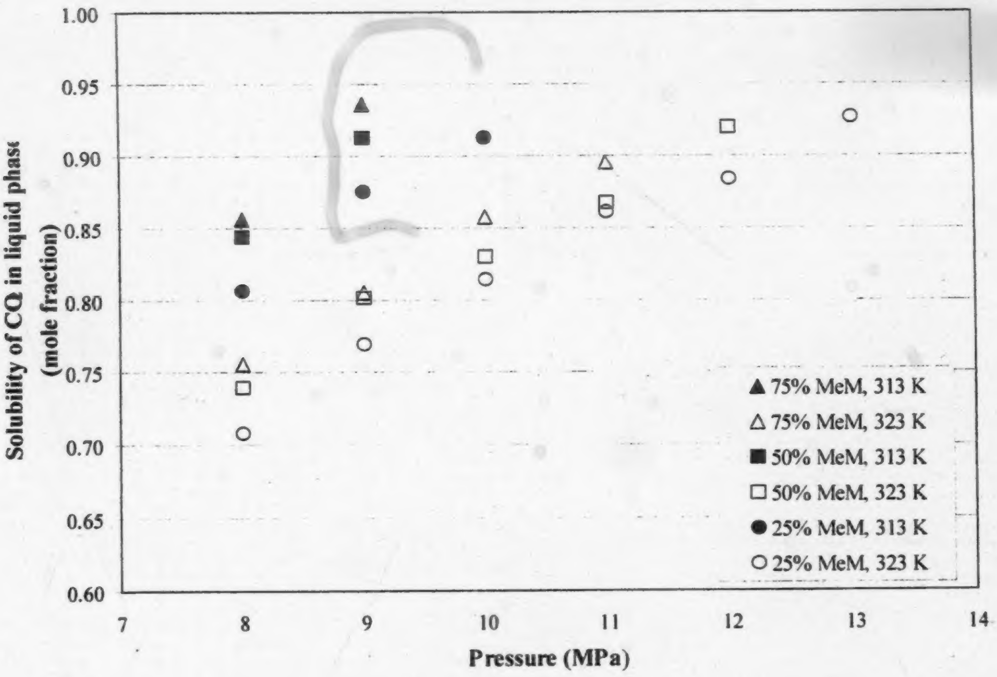


Figure 5.27. Solubility isotherms of CO_2 in MeM/MeP mixture (Data from Ref. 8).

5.3.2.3. Partition coefficients

The dependence of partition coefficients on mixture composition is of special interest in countercurrent separation. In a fractionation column, operating isobarically and isothermally, the change in the composition of vapor and liquid phases over the height of the column is the only factor that determines the extent of separation. Similar to the TG systems, partition coefficient trends were observed to deviate from solubility trends qualitatively as well as quantitatively. Different trends were observed for OA in the FA system (System 5), for the MeO/MeL ester system (System 7) and OA in FA/FAME system (System 9). While solubility diminution was observed for OA in all OA/LA mixtures (Fig. 5.16), a significant enhancement of OA partition coefficient was observed in 15% OA/85% LA mixtures at both temperatures (Fig. 5.28). For the MeO/MeL system, solubilities were dependent on feed concentration (Fig. 5.17) whereas partition coefficients were nearly independent of feed concentration in the ternary system (Fig. 5.29). In the OA/MeO system, solubility enhancement was observed only for OA in 25% OA/75% MeO mixture at 313 K (Fig. 5.18), whereas the partition coefficients at all conditions were enhanced compared to those of binary system and the level of enhancement decreased with increasing feed concentration (such that enhancement was higher at lower OA feed concentrations) (Fig. 5.30). At 333 K, partition coefficient of OA was enhanced in the 50% OA/50% MeO and 25% OA/75 % MeO mixtures, whereas 75% OA values were similar to binary values.

Solubility diminution of an individual component with the addition of a second solute has been observed when the solute mixture was liquid under extraction conditions [9, 20] and this has been attributed to the decrease in vapor pressure due to the presence

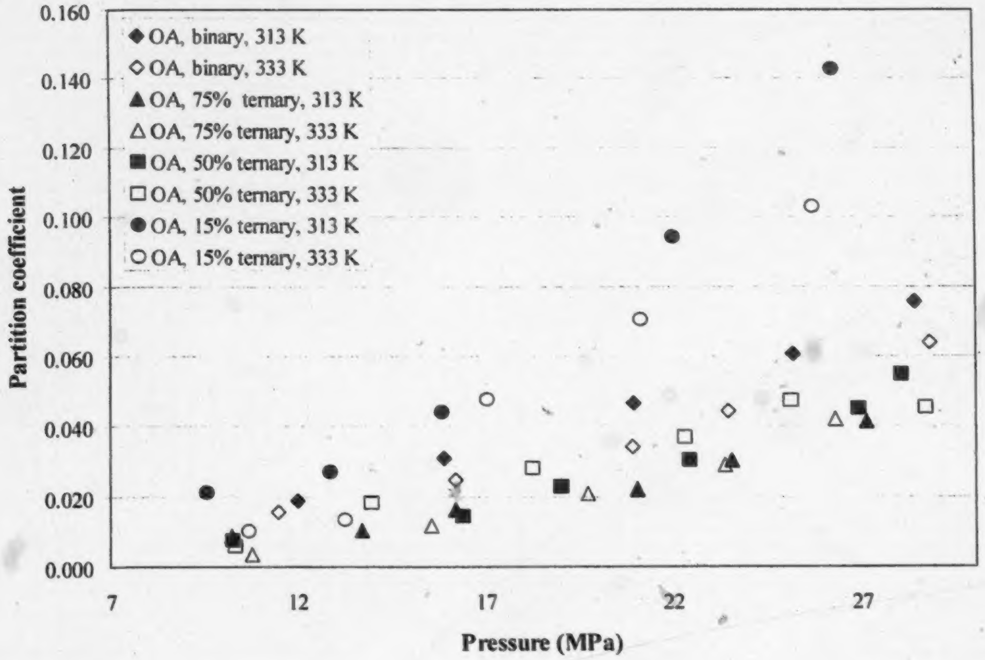


Figure 5.28. Binary and ternary (OA/LA) partition coefficient isotherms of OA in $SCCO_2$ (Data from Refs. 6 and 7).

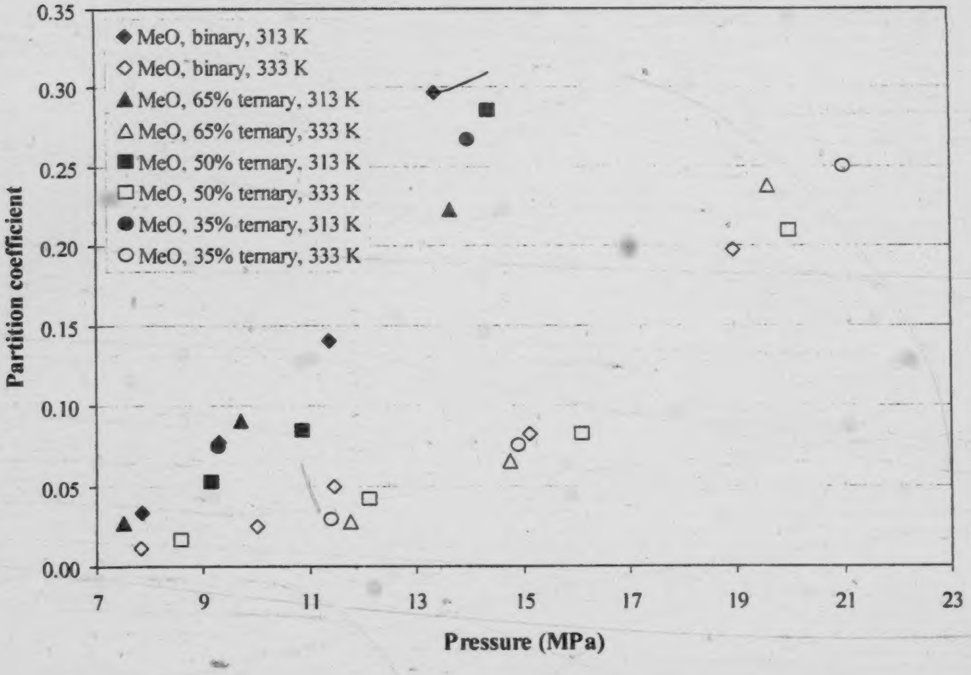


Figure 5.29. Binary and ternary (MeO/MeL) partition coefficient isotherms of MeO in $SCCO_2$ (Data from Refs. 6 and 7).

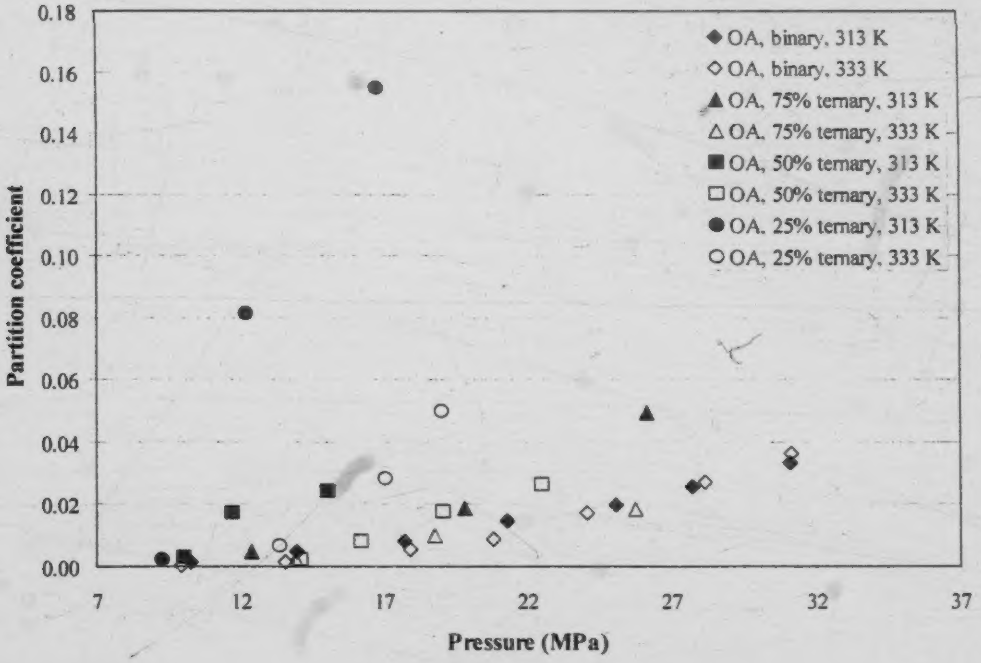


Figure 5.30. Binary and ternary (OA/MeO) partition coefficient isotherms of OA in $SCCO_2$ (Data from Refs. 9 and 10).

of another solute. Raoult's law states that the vapor pressure of a mixture component is proportional to its concentration in the mixture. The increase in solubility diminution with decreasing feed concentration is thus expected, as vapor pressure will decrease with decreasing feed concentration.

The solubility enhancement observed in FAE mixtures might be due to the high solubility of esters (highest among the studied compounds, in the range of 10^{-2}-10^{-5} mole fraction), which results in a high concentration in the supercritical phase. However, it should also be noted that as the solubility of FAE in $SCCO_2$ are relatively high, most of the solubility measurements are carried out at low pressures close to the critical point, where the effect of operating conditions on solubility and any potential for experimental error is most pronounced. Solubility enhancement has been reported in L-V systems for solutes that exhibit S-V equilibria in the binary system [15].

While interpreting the trends observed in L-V systems, the effect of the presence of a second solute on the intermolecular interactions in the liquid phase should also be considered. In cosolvent systems, the intermolecular interactions between the cosolvent and the solute are the main contributors to the cosolvent effect. However, the interactions between the cosolvent and the solute in the condensed phase may also affect the solubility behavior as observed in sterol-alcohol systems where formation of complexes in the solid phase decreased the cosolvent effect [27]. In ternary or higher systems, the concentration of the other solute(s) is usually substantially higher than the concentration of a cosolvent in the sample. Therefore, the intermolecular interactions in the liquid phase are expected to have a more pronounced effect on the solubility behavior. Thus, the effect of the presence of another solute on the intermolecular interactions in the liquid phase as well as the supercritical phase should be considered while interpreting the observed trends.

Addition of a second solute to a binary system may also have a dilution effect and decrease the strength of interactions between other solute molecules. This will have a significant effect on solubility behavior (might result in solubility enhancement of these solutes) if there are relatively strong interactions between solute molecules such as H-bonding. Solubility enhancement observed for OA in the presence of MeO and OOO may be explained by this dilution effect. OA is a polar compound, which can participate in H-bonding due to its carboxyl group. It can self-associate as the carboxyl group can act both as a H-bond donor and acceptor. The presence of MeO and OOO in high concentration, which are less polar than OA due to the esterification of the carboxyl group, will decrease the intermolecular interactions between OA molecules. Nilsson et al. [12] attributed the enhancement observed in partition coefficients of MO and DO in the presence of OOO in the quaternary mixture (Figs. 5.31-5.33) to this dilution effect. The presence of OOO increased the partition coefficients of MO and DO, which are polar compounds, and similar to OA can H-bond with themselves and each other due to the presence of carboxyl groups. Solubility enhancement in these systems will increase with concentration of the diluting solute. This concentration effect was clearly observed in the quaternary glyceride mixture with increasing concentration of OOO (Figs. 5.31-5.33).

Similarly, the effect of feed composition on CO_2 solubility can be explained by the effect of mixture composition on intermolecular interactions in the liquid phase. As indicated above, the solubility of CO_2 decreased with increasing MeO concentration in OA/MeO and LA concentration in OA/LA mixtures such that CO_2 solubility increased

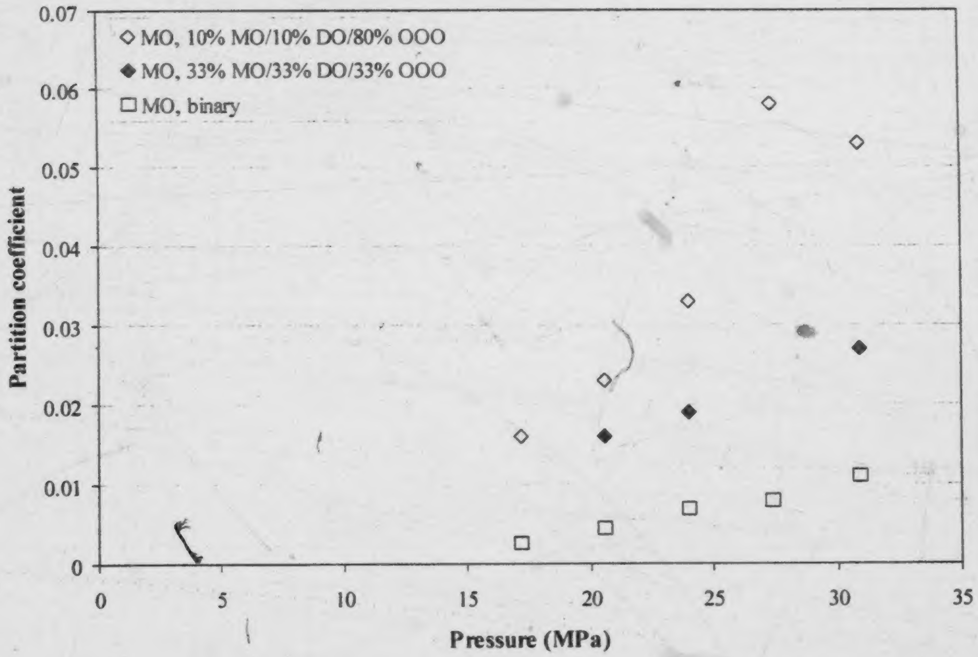


Figure 5.31. Binary and quaternary (MO/DO/OOO) partition coefficient isotherms of MO in $SCCO_2$ (Data from ref. 12).

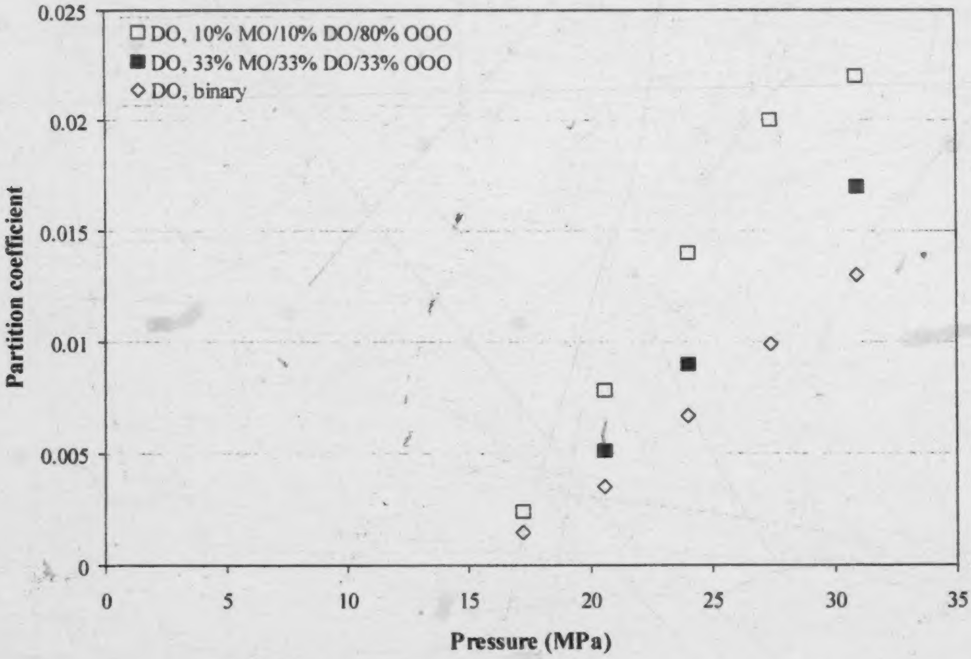


Figure 5.32. Binary and quaternary (MO/DO/OOO) partition coefficient isotherms of DO in $SCCO_2$ (Data from ref. 12).

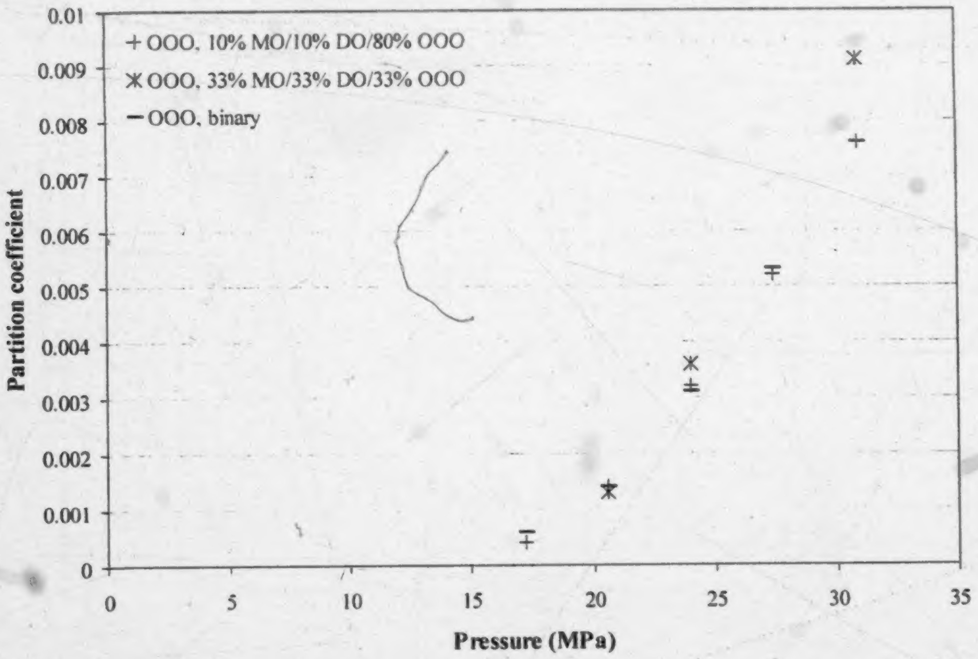


Figure 5.33. Binary and quaternary (MO/DO/OOO) partition coefficient isotherms of OOO in $SCCO_2$ (Data from ref. 12).

with concentration of the diluting compound, presence of which decreased solute-solute interactions in the liquid phase. In FAE mixtures, CO_2 solubility was independent of feed concentration (System 7) or increased with the concentration of the more soluble ester (System 8).

5. 3.3. Implications for fractionation

Information on the effect of operating conditions and feed composition on separation efficiency in the model systems studied have important implications for fractionation of complex lipid mixtures. For solid mixtures, further purification of the extract under saturation conditions is not possible as the vapor phase concentration is independent of feed composition. When the initial solid mixture is extracted from another source using $SCCO_2$, the more soluble solute will be enriched by a factor determined by selectivity. However, if the extracted solute mixture is precipitated in a separator and again put into contact with fresh CO_2 under the same conditions, no further separation can be expected. Thus, cascade processing is not a viable option unless the composition can be changed by modifying the solvent properties (for example, by changing the operating conditions) [18]. For liquid mixtures, the separation efficiency can be enhanced by introducing an external or internal reflux as vapor phase concentration is dependent on feed composition. The effect of operating conditions on separation efficiency together with their effect on solvent requirements should be known for the design of fractionation processes.

Selectivity values in ternary mixtures were calculated as the ratio of partition coefficients of solutes of interest and used to determine the effect of feed concentration and operating conditions on separation efficiency. Selectivities were also calculated using binary solubility values to determine the predictive ability of binary data.

In the FA system (OA/LA) (Fig. 5.34), highest selectivity (1.5-2.4) was observed for 15% OA/85 % LA mixture due to the high partition coefficient of OA. Selectivities were in the range of 0.92-1.08 for the 50% mixture, 0.70-1.02 for the 85% OA mixture indicating enrichment of LA in the extract. Selectivity in the 15% OA mixture was observed to increase with pressure and the temperature effect was dependent on pressure such that selectivities at 333 K were higher at pressures ≤17 MPa. For the remaining mixtures, it was not possible to establish trends with pressure and temperature. The FA model mixture indicates that fractionation of FA based on unsaturation can be achieved, but further research is required to confirm these high selectivity values.

$SCCO_2$ fractionation of FA generally involves the conversion of FA to more stable and more soluble fatty acid alkyl (methyl or ethyl) esters. In a rare study on column fractionation of FA in palm oil deodorizer distillate, Machado and Brunner [28] reported an enrichment of palmitic acid from 52.5 wt% to 74.4 wt% in the extract, whereas OA+LA were enriched from 46.27 wt% to 59.0 wt% in the raffinate.

In the MeM/MeP ester mixture, selectivity values were highest for 12.5% MeM mixture (3.7-1.4 and 3.6-1.2) at both 313 and 323 K. At 313 K it was followed by 25% MeM mixture (1.6-1.2); selectivity values for the rest of the mixtures were in the range (1.0-1.3). Pressure dependence of selectivity values was dependent on feed concentration. For example, while the selectivity in the 50/50 mixture increased with pressure at 323 K, it decreased with pressure for 12.5% MeM/87.5% MeP mixture at both temperatures. Trends of temperature dependence of selectivity values for MeM/MeP system could not

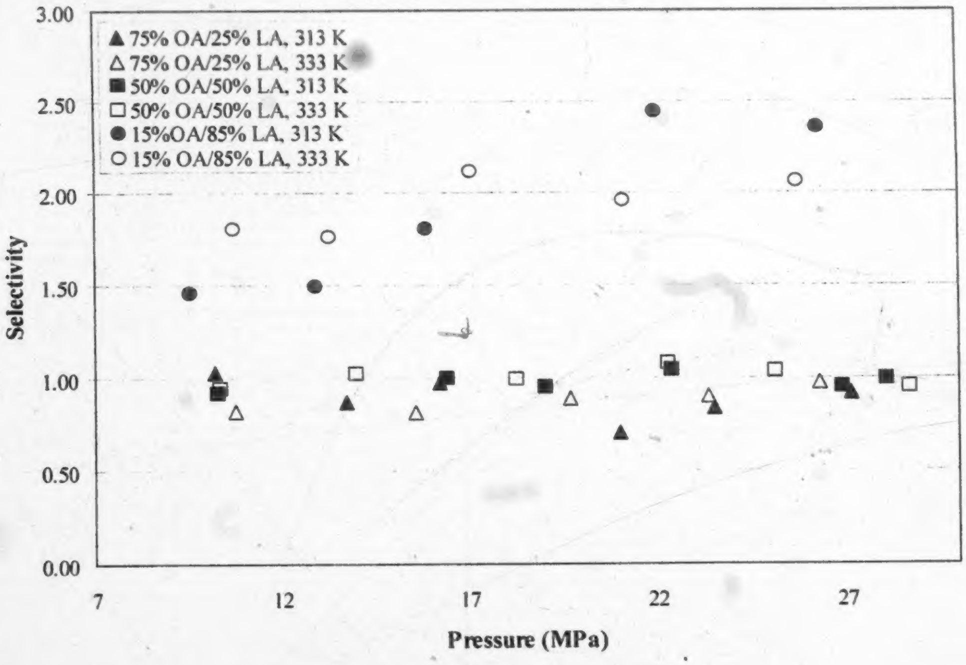


Figure 5.34. Selectivity (OA/LA) in system 5 (Data from ref. 6).

be established. Selectivities were around one in the ester system, MeO/MeL (0.94-1.06) (Fig. 5.35). Supercritical CO_2 fractionation of FAE mixtures has been widely investigated as a means for the concentration of ω-3 FA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have received increasing attention in the last decades due to mounting evidence on their health benefits [29-32]. As suggested by the above selectivity values, mixture components could be fractionated by carbon number, but no separation by degree of unsaturation could be achieved [29-32].

For the FAME/FA system, highest selectivities (8.7-9.5) were achieved at the lowest pressures tested at 313 K (Fig. 5.36). For the 25% MeO mixture, selectivity values decreased with pressure at this temperature whereas for 75% MeO + 25% OA and 50% MeO + 50% OA mixtures an initial large drop with pressure (selectivities dropped to 0.8 and 2.3, respectively, when pressure was increased to 12 MPa) was followed by a slight increase. At 333 K, selectivities were in the range of 1.2-4.9 and increased slightly with pressure in 25% and 75% MeO mixtures, whereas a minimum was observed in the 50% mixture. Selectivities increased with decreasing MeO concentration in the feed at both temperatures.

In the OA/OOO mixture (Fig. 5.37), the selectivities in the 30% OA/70% OOO mixture were highest (16.8-2.8 at 313 K and 18.0-2.8 at 333 K) and showed a linear decrease with pressure. The extent of separation achievable in this system merits further consideration with a special focus on its implications for the deacidification of vegetable oils using $SCCO_2$. Supercritical deacidification of vegetable oils has been investigated for a variety of oils such as olive oil [5, 33-37], peanut oil [38], rice bran oil [39] and black cumin oil [40] with promising results. As noted by others [33], the concentration range

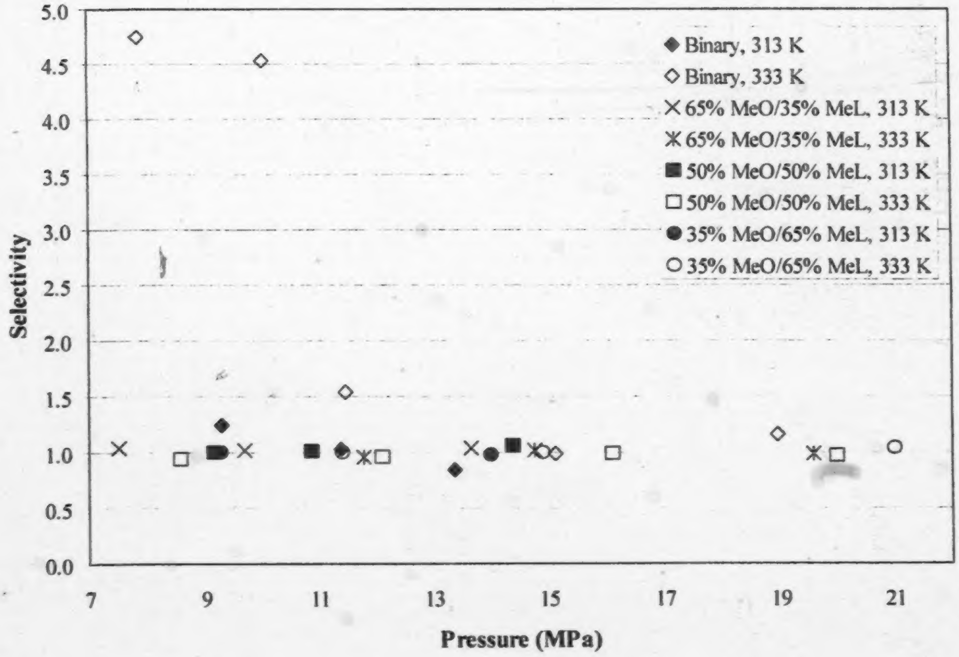


Figure 5.35. Selectivity (MeO/MeL) in system 7 (Data from ref. 6).

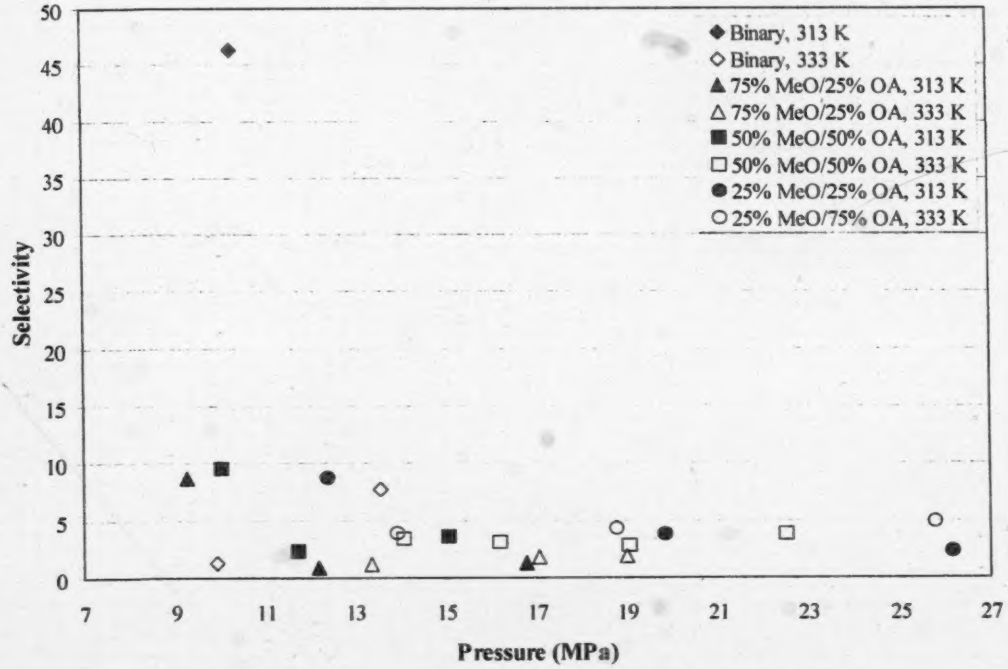


Figure 5.36. Selectivity (MeO/OA) in system 9 (Data from ref. 9).

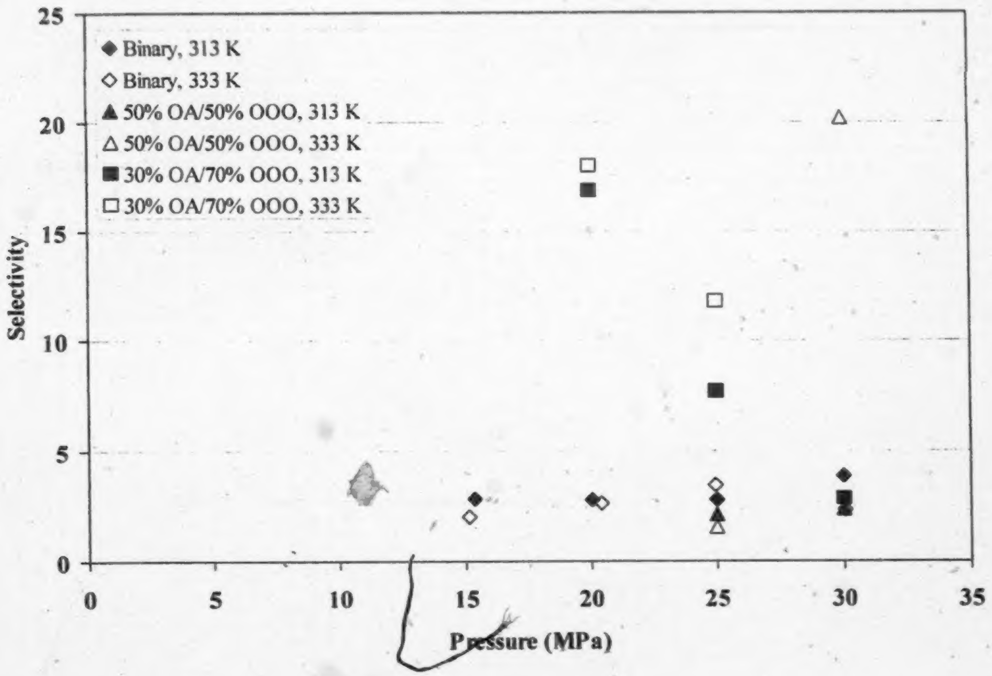


Figure 5.37. Selectivity (OA/OOO) in system 10 (Data from ref. 11).

used this study is higher than that of crude vegetable oils, where the free fatty acid concentration can be as high as 7% of the oil. The high selectivity values obtained in this system however merit further attention and can have important implications for the fractionation of lipid mixtures.

Selectivities were in the range of 45.6-416.0; 10.6-17.2 and 2.4-6.7 in LLL/PPP, MMM/PPP and LLL/MMM mixtures, respectively (Fig. 5.38). Selectivities decreased with pressure in LLL/PPP mixture and the highest value was achieved at the lowest pressure for LLL/MMM mixture. In the quaternary LLL/MMM/PPP mixture, the selectivity values for LLL/PPP and MMM/PPP were in the range of 12.5-40.9 and 6.4-13.8, respectively (Fig. 5.39). The selectivity values decreased with pressure in the pressure range of 9.2-15 MPa. Selectivity values for LLL/MMM were in the range of 2-3. Ternary selectivity values were higher than those obtained in the quaternary mixture. In the quinary TG mixture (System 13), the selectivity for PPP/OOO was in the range of 1.1-1.4 and 1.4-1.7 at 313 and 333 K, respectively.

In the glyceride mixture investigated by Nilsson et al. [12], selectivities were in the range of 1.6-6.7 for MO/DO; 3.0-39.0 for MO/OOO and 1.9-5.9 for DO/OOO and decreased with pressure (Fig. 5.40). In this system, selectivity values increased with decreasing concentration of MO and DO and increasing OOO concentration. Increase in selectivity with OOO concentration can have important implications for fractionation of glyceride mixtures (such as reaction products); therefore, investigation of a wider concentration range is warranted to establish trends and determine concentration levels that can be exploited for fractionation purposes. $SCCO_2$ column fractionation of glyceride

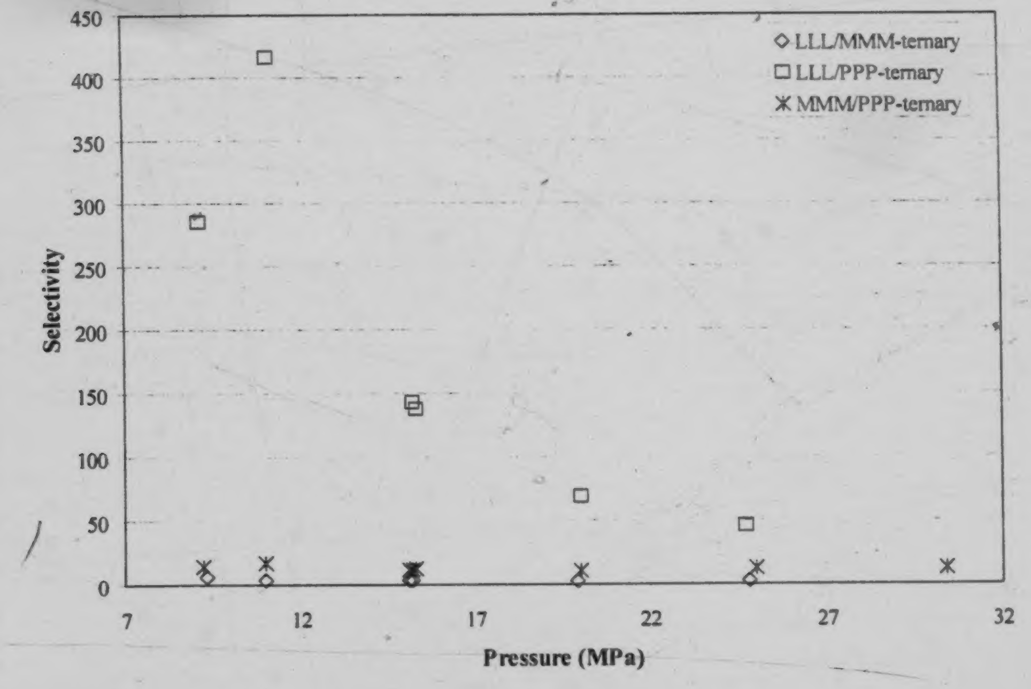


Figure 5.38. Selectivities (LLL/MMM, LLL/PPP, MMM/PPP) in ternary triglyceride systems (Systems 1-3) (Data from ref. 4).

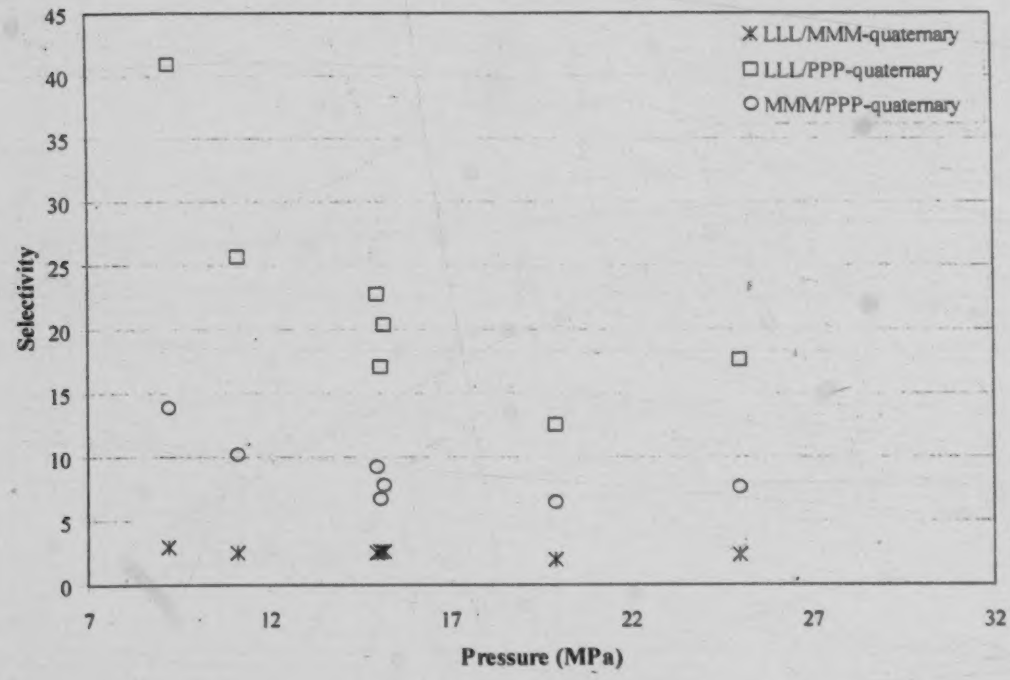


Figure 5.39. Selectivities (LLL/MMM, LLL/PPP, MMM/PPP) in quaternary triglyceride system (System 11) (Data from ref. 4).

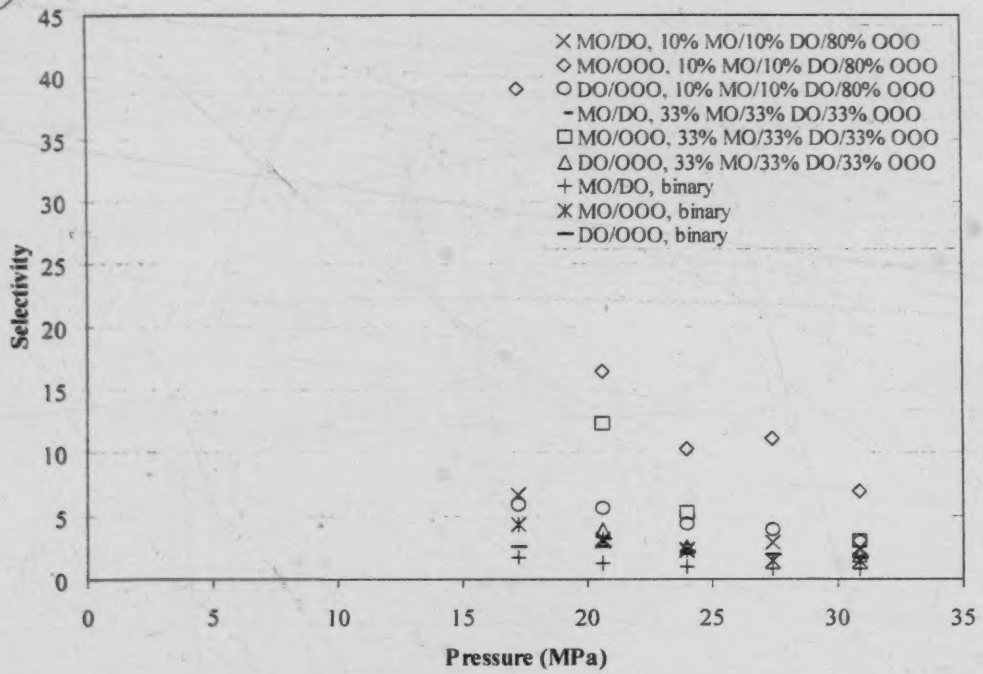


Figure 5.40. Selectivities (MO/DO, DO/OOO, MO/OOO) in quaternary glyceride mixture (System 12) (Data from ref. 12).

mixtures (MG, DG, TG) for the enrichment of MG yielded products with a composition comparable to that obtained by molecular distillation [41].

A decrease in selectivity with pressure has been observed during fractionation of a variety of mixtures [29, 41, 42] and has been attributed to the differences in the pressure dependence of solubility of mixture components [41]. On their study on the solubility of milk fat TG, Arul et al. [42] explained this observation by the relative effect of pressure on vapor pressure and hence solubility of lipid components. They stated that the isothermal increase in vapor pressure with pressure will be more pronounced for less volatile molecules (longer chain molecules in a homologous series because of their higher molar volume). Therefore, as pressure increases the solubility of the more volatile/soluble component increases to a lesser extent, leading to decreasing selectivity values. However, the effect of pressure on the vapor pressures of solutes, especially the low volatile ones such as TG, is not likely to have a significant effect on solubility behavior. The opposing trends observed for selectivity and extraction rate/solvent loading pose a processing challenge and necessitate the optimization of operating conditions with respect to these two parameters. The opposite trend, increasing selectivity with pressure has also been observed for a few systems such as palm oil deodorizer distillate (in a phase equilibrium study), where the solubility in vapor phase and selectivity values both increased with pressure and temperature [43] and in the deacidification of olive oil (in a countercurrent column fractionation study) [34] and requires further attention.

Differences in the temperature dependence of solubilities/partition coefficients of mixture components can also be exploited to achieve separation. The existence of a crossover region in multicomponent systems, where a temperature change will affect the solubility of mixture components in opposing ways has been proposed as a means to separate solid mixtures [44]. However, in liquid mixtures the separation efficiency will be determined by the partition coefficients of the mixture components. Even if the crossover behavior persists in liquid mixtures as in solid mixtures, the partition coefficients may not follow similar trends. The exploitation of differences in the temperature dependence of partition coefficients of mixture components in column fractionation with a temperature gradient has not been explored and requires further attention. In a column fractionation with a temperature gradient, less soluble components drop out of solution as the feed mixture moves up in an ever increasing temperature gradient as the column is operated in the retrograde region (solubility decreases with temperature). The separation efficiency would increase if the solubility/partition coefficient of the most soluble component is increased and that of the others decrease with temperature. The crossover range however cannot be exploited for the separation of mixtures, if all components exhibit retrograde solubility behavior (such as fractionation of FAE), or when the more soluble solute shows retrograde behavior over the entire experimental range. For example, in the TG mixture PPP/OOO/PPO/POO, the crossover of PPP appeared to occur at a lower pressure than the rest of the mixture components. In this crossover pressure range, a temperature increase will increase the partition coefficient of PPP, whereas it will decrease that of OOO. The selectivity of the separation will thus be improved as partition coefficient of PPP will be greater than that of OOO.

Comparison of binary and multicomponent solubility behavior of lipid components provides valuable information on the trends of solubility behavior observed in multicomponent systems, which adds to our understanding of the nature and extent of deviations from binary behavior. Binary solubility data may not be predictive of the separation efficiency as multicomponent trends may be different from binary trends both qualitatively and quantitatively. If the solubility of the less soluble compound is enhanced over its binary value in the mixture, as in the extraction of ternary solid mixtures, a less favorable separation will result compared to the separation predicted from binary solubility data. Separation efficiency will be improved if the partition coefficient of the more soluble component is enhanced in the multicomponent mixture as observed in the quaternary glyceride mixture, resulting in selectivities higher than those predicted by binary data (Fig. 5.41). Separation may also be improved in S-L-V systems if the more soluble component exists as the excess solute [15], since solubility enhancement of the excess solute is accompanied by solubility diminution of the other solute in these systems [15]. However, melting of the less soluble component in the mixture may result in separation efficiency considerably lower than that predicted by binary data as observed for PPP/OOO in the quinary TG mixture. OOO/PPP binary selectivity values were in the range of 7.5-18.4 at 313 K and 1.4-1.6 at 333 K, whereas ternary values were in the range of 1.1-1.4 and 1.4-1.7 at 313 and 333 K, respectively.

For the ternary TG systems, the binary solubilities yielded higher selectivity values (Figs. 5.38, 5.41), but the extent of deviation differed between systems. For LLL/PPP ternary mixture, selectivities were lower than binary ones at pressures>15 MPa. For the MMM/PPP system, the binary solubilities yielded higher selectivity values in the range of 17.7-28.1, which decreased with pressure. Pressure dependence was also different in some systems; for example, binary selectivity values for LLL/MMM increased with pressure, whereas ternary values was highest at the lowest pressure

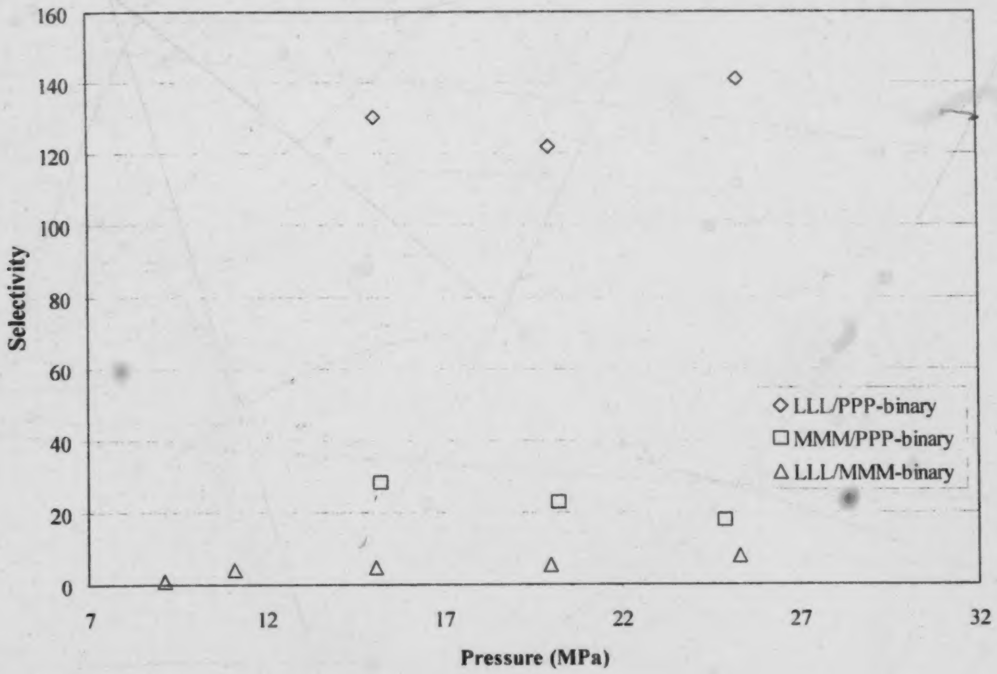


Figure 5.41. Selectivities (LLL/MMM, LLL/PPP, MMM/PPP) predicted using binary data (Data from ref. 4).

investigated. In L-V systems, binary selectivity values were higher for MeO/MeL, MeO/OA, OA/OOO systems (Figs. 5.35-5.37). For the MeM/MeP system, binary selectivity values were higher than ternary values at 313 K, whereas higher values were obtained at 323 K.

Binary solubility data can be used as a basis for the design of fractionation of multicomponent mixtures (for the selection of fractionation conditions) for which the effect of operating conditions on solubility behavior will not vary to a significant extent as demonstrated by Liang and Yeh [45] for FAE. However, the presence of compounds of different lipid classes and phase change will complicate the phase behavior. Hence, caution should be exercised when using binary data for the design of fractionation processes.

5.4. SUMMARY

Knowledge of phase behavior information is essential for the design of any supercritical process. Study of simpler binary systems and model lipid mixtures will provide valuable information on the effect of operating conditions and mixture composition on solubility behavior and separation efficiency and the nature and extent of deviation from binary behavior. The solubility behavior in multicomponent systems can be categorized based on the existing phase equilibria; therefore, information on the melting behavior of the mixtures is essential for accurate interpretation of data. In multicomponent mixtures, intermolecular interactions in the liquid phase may also have a significant effect on solubility behavior and therefore should be considered.

The effect of operating conditions on partition coefficient and selectivity should be known for the optimal design of fractionation processes. In systems where pressure has an opposite effect on solvent loading and selectivity values, operating conditions should be optimized considering these two factors. Differences in the temperature dependence of partition coefficients of mixture components can be exploited for the (column) fractionation of mixture components.

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6. COLUMN FRACTIONATION OF CANOLA OIL DEODORIZER DISTILLATE USING SUPERCRITICAL CARBON DIOXIDE[1]

6.1. INTRODUCTION

Supercritical carbon dioxide ($SCCO_2$) has been widely used in the last decade for the processing of fats and oils for fractionation purposes. While fractionation can be achieved during or after the extraction step using fractional extraction or fractional separation, fractionation of liquid feed material is usually carried out in packed columns, which are custom built in-house or by manufacturers of extraction units.

Vegetable oil deodorizer distillate, which is a by-product of the conventional refining process, is a very complex mixture containing aldehydes, ketones, free fatty acids (FFA), glycerides and bioactive components such as sterols and tocopherols and has been used commercially for the production of these components [1, 2]. Processing of distillates using traditional methods and supercritical fluid (SCF) technology has been presented in Chapters 1 and 3. Although the concentration of sterols and tocopherols from soybean deodorizer distillate [3-6] and squalene from olive oil distillate [7] have been investigated, studies on canola oil distillate have been scarce. Ramamurthi et al. [8] and Ramamurthi and McCurdy [9] studied the esterification and subsequent processing (vacuum distillation) of soybean and canola oil deodorizer distillates and noted that canola oil distillate can be used as a cheap source of fatty acids (FA) due to its high FFA and low sterol and tocopherol contents. $SCCO_2$ fractionation of canola oil distillate has not been reported.

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[1] A version of this chapter is to be submitted to the Journal of the American Oil Chemists' Society for consideration for publication.

Value-added processing of canola oil deodorizer distillate will benefit the canola oil processing industry in Canada considerably as canola is the leading oilseed crushed and refined in Canada [10] and canola oil deodorizer distillates are either added back to canola meal for use as animal feed or sold to leading manufacturers of sterols and tocopherols for further processing.

Therefore, the objective of this study was to study the column fractionation of canola oil deodorizer distillate using $SCCO_2$ to determine the feasibility of value-added processing of this feed material. The specific objectives were: a) to design and build a $SCCO_2$ fractionation column, and b) to determine the effect of operating conditions (temperature and pressure) on fractionation efficiency.

6.2. MATERIALS AND METHODS

6.2.1. Materials

Canola oil deodorizer distillate was supplied by CanAmera Foods (Wainwright, AB). All lipid standards used for gas chromatography (GC) analysis were from Sigma Chemical Co. (Oakville, ON) and 99% pure unless otherwise noted. HPLC grade hexane and chloroform and ACS grade pyridine were obtained from Fisher Scientific (Fair Lawn, NJ). The derivatization agent, Sylon BFT (N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA): Trimethylchlorosilane (TMCS), 99:1) was from Supelco Inc. (Bellefonte, PA) and ethanol (100%) was supplied by Commercial Alcohols Inc. (Winnipeg, MB). Liquid CO_2 (bone dry) used in the fractionation experiments and GC gases, air, helium, hydrogen and nitrogen were from Praxair Canada Inc. (Mississauga, ON).

6.2.2. SCCO$_2$ fractionation column

A SCCO$_2$ fractionation column was designed and built in-house (Fig 6.1). The column was not designed for a specific application but rather to accommodate SCCO$_2$ fractionation of a wide range of feed materials. Operational flexibility (ability to make modifications) was a major consideration during the design of the column. The column was designed based on previously reported systems and is similar to other columns operational around the world (in Japan [11], USA [12] and Europe [13]). The design phase included literature review, determination of column dimensions and layout and identification of necessary components (high pressure column components: stainless steel tubing (2.54 cm (1") o.d. for the column and 0.635 cm (1/4") o.d. for sampling, feed, CO$_2$ and raffinate lines), safety head, sheath–type thermocouple, connectors (tees, crosses), adapters, couplings, valves (metering and needle valves); heating elements: heaters, temperature controller, temperature readout, thermocouples). The maximum pressure limit was chosen to be 68.9 MPa, considering the practical operating range for fractionation operations. Therefore, all column components are rated at 68.9 MPa. All column components were manufactured by Autoclave Engineers Inc. (Erie, PA) and purchased from Zimco Valves and Tubings (Calgary, AB) and all electrical/heating components were supplied by Tru-temp Electric Heat Ltd. (Edmonton, AB). Technical support services provided assistance in putting the parts together according to our design. Preliminary trials were carried out to test the heating and pressurization performance of the column, to determine the method of feed introduction, to determine the optimum cleaning procedures after a test run and to finalize the operating protocol.

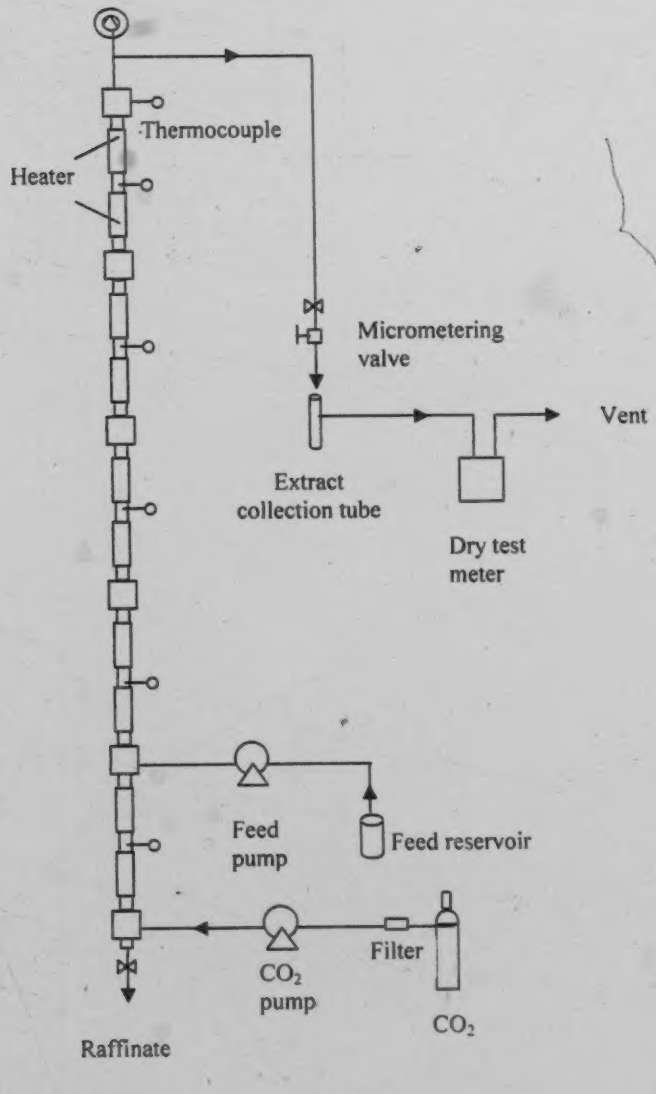
Thermocouple
Heater
Micrometering valve
Vent
Extract collection tube
Dry test meter
Feed pump
Feed reservoir
Filter
CO_2 pump
CO_2
Raffinate

Figure 6.1. Schematic diagram of $SCCO_2$ fractionation column.

The column (3.1 m) is made up of five sections (2.54 cm (1") o.d., 1.75 cm (0.688") i.d.), which are connected through cross or tee fittings. The 0.635 cm (1/4") processing lines for feed, CO_2 introduction and sampling are connected to the column through these fittings. The column is packed with 0.41 cm (0.16") stainless steel Propak packing material (Cannon Instrument Company, State College, PA). An ISCO syringe pump (260D, Perkin Elmer Life and Analytical Sciences, Woodbridge, ON) is used to feed CO_2 to the column from the bottom zone. Two sampling lines are connected at the top and bottom of the column to collect extract and raffinate samples, respectively. The sampling lines contain on/off valves, whereas a metering valve is also added to the extract line for manual control of the flow rate. A pressure gauge is used at the top of the column to monitor column pressure.

Each section is heated by two band heaters wrapped around the column wall and connected to a separate temperature controller, which read the temperature from a thermocouple (Type J) placed on the outer wall in the middle of each section. In this manner, temperature of each section can be controlled separately and a temperature gradient can be maintained along the column. An internal reflux can thus be created by establishing an increasing temperature profile along the column provided that the solubilities of the feed components decrease with increasing temperature in the experimental range. As the mixture moves up the column, the least soluble components will drop out of the solution while CO_2 stream will be enriched in the more soluble components. A sheath-type thermocouple connected to a temperature read-out is used at the top of the column to monitor the fluid temperature. CO_2, feed and sampling lines and the expansion valves can be heated using Glasrope heaters connected to rheostats to avoid condensation on the inside walls of the tubing and freezing of expansion valves.

6.2.3. Experimental design and operating protocols

Semi-continuous fractionation of canola oil deodorizer distillate was carried out to determine the effect of pressure (20, 25 MPa at 70-100 °C), temperature and a linear temperature gradient (70 °C, 100 °C, 70-100 °C at 25 MPa) on fractionation yield and separation efficiency. In a typical run, the column was heated and pressurized with CO_2 after 25 mL of melted feed material was introduced into the column using a peristaltic pump (Watson-Marlow 320 F, Watson-Marlow Bredel Inc., Wilmington, MA). The amount of feed introduced into the column corresponded to approximately 1/5 of the internal void volume of one column section. Therefore, no carry over of the feed material due to CO_2 flow was expected.

After the desired temperature and pressure conditions were achieved, the extract valve was opened and CO_2 flow thus started. CO_2 stream leaving the column at the top was expanded to atmospheric pressure through a heated valve while the extract was collected into side-armed glass tubes immersed in an ice water bath. CO_2 passed through a gas meter to measure its volume and was then vented. Six fractions were collected in each run (1 fraction/30 min), which lasted a total of 3 h. However, as no sample was collected in the first half hour in any of the runs, data for five fractions were reported (such that the first fraction corresponds to 1 h of fractionation). This time lag is attributed to the residence time of CO_2 in the column, which is dependent on CO_2 flow rate and column volume (determined by column dimensions). It is estimated that the amount of

CO_2 pumped through the column over a 30 min period corresponded to 40-60% of the internal void volume of the column depending on the operating conditions.

The yield of each fraction was determined gravimetrically. The fractions were transferred into a sample vial using hexane, which was evaporated under a gentle flow of N_2 after the transfer. CO_2 flow rate was maintained at 3±0.3 L/min (measured at ambient conditions) using the micrometering valve. After the last fraction was collected, the column was depressurized and the raffinate/residue was collected using the raffinate valve at the bottom of the column during depressurization. After each run, the column was cleaned by pumping ethanol (200 mL) first and then by pumping CO_2 at 20-25 MPa and 70 °C with an additional 30 mL ethanol for 3 h.

One of the major processing challenges encountered was the introduction of the feed into the column. The distillate was a viscous paste at room temperature, and therefore had to be heated prior to each run to ensure melting. Furthermore, the feed line had to be heated to prevent solidification. However, the available HPLC-type feed pumps were not able to pump the feed without solidification. Therefore, a peristaltic pump had to be used with its inherent difficulties for feed introduction. The column was heated prior to feed introduction and an additional heater was used on the column fitting where feed was introduced.

6.2.4. Compositional analysis

Compositional analysis of the feed, residue and fractions obtained at different operating conditions were carried out using a gas chromatograph (Varian 3600, Varian Canada Inc., Mississauga, ON) equipped with a capillary DB-5 HT column (30 m, 0.25 mm id, 0.1 μm film thickness; J&W Scientific/Agilent Technologies, Palo Alto, CA) and flame ionization detector. A GC method reported by Verleyen et al. [14] was adopted after modifications to ensure the elution of triglycerides (TG). Details of the temperature profile of the GC method are presented in Table 6.1. This method enabled the analysis of all the components of interest (FFA, mono-, di- and triglycerides (MG, DG, TG), sterols and tocopherols). Fatty acid composition of the canola oil deodorizer distillate was determined using a SP 2560 capillary column (100 m, 0.25 mm id, 0.2 µm film thickness; Supelco, Bellefonte, PA) according to procedures outlined by Yurawecz et al. [15]. FFA content of the feed was determined by titration using AOCS Method Ca 5a-40 [16].

6.2.4.1. Preparation of sample and standards

Samples and standards were derivatized with Sylon BFT prior to GC analysis to increase their volatility. An aliquot of sample (20 mg) was weighed into a test tube and dissolved in 0.5 mL pyridine. After addition of 0.5 mL Sylon BFT, the tube was placed in an oven at 70 °C for 20 min for the completion of the derivatization reaction. Individual standards (10 mg) were derivatized using the same procedure. The mixture was then diluted with the addition of 9 mL chloroform. Standard solutions were prepared by transferring 0.05 mL aliquots of each standard into a GC vial and adding 1 mL of chloroform. Sample (0.5 mL) was transferred to a GC vial for analysis where it was diluted by the addition of 0.5 mL chloroform.

6.2.4.2. Qualitative analysis

Qualitative analysis was carried out by comparing the retention times of standards with those of mixture components. Standard mixtures were analyzed in parallel with every batch of sample (12 vials/batch). Typical chromatograms for the standard mixture and the sample are given in Figures 6.2 and 6.3, respectively. Deodorizer distillate is a

Table 6.1. Parameters of the GC Method used in the analysis.

	Temperature (°C)	**Hold time (min)**	**Ramp rate (°C/min)**
Column Program			
Initial	60	2	
1	140	0	30
2	235	7	5
3	350	3	5
4	380	14	10
Injector Program			
	70	0.1	
	370	69.0	150
Detector			
	370	-	-

very complex mixture of more than 100 components of various lipid classes such as FA, MG, DG, TG, tocopherols and sterols. Every major lipid class in turn contains a range of components, usually varying in carbon number, which further increases the complexity of the mixture. Due to the wide range in their volatility, peaks of components belonging to different lipid classes overlap, complicating the analysis. Therefore, qualitative analysis of the mixture using single standards as representatives of each lipid class is not sufficient. Information on the FA composition of the mixture (Table 6.2) can be used to determine the standards to be used for the qualitative analysis. In light of these results, C16 and C20 glycerides (Nu-Chek-Prep Inc., Elysian, MN) were used to identify the FFA and glyceride (MG, DG, TG) groups. A sterol standard mixture containing campesterol, stigmasterol, sitosterol and brassicasterol (Matreya Inc., Pleasant Gap, PA); α-, β-, γ- and δ-tocopherol and squalene standards were also used for the qualitative analysis of the minor components. The unidentified components with retention times less than that of C16 FFA were grouped and reported as "volatiles". Using this approach, 95-97% of sample components were accounted for.

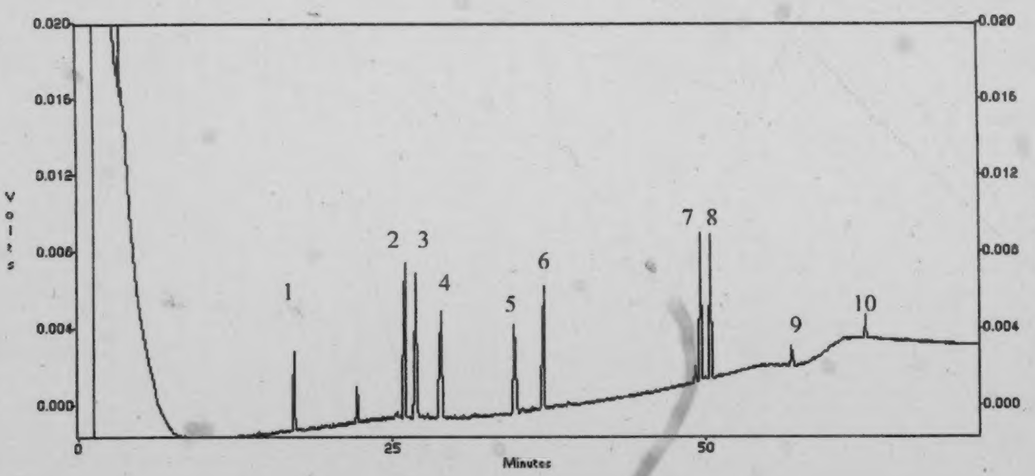

Figure 6.2. A typical GC chromatogram of the derivatized standard mixture (1 = oleic acid, 2 = monoolein, 3 = squalene, 4 = δ-tocopherol, 5 = α-tocopherol, 6 = stigmasterol, 7 = diolein, 8 = internal standard, 9 = cholesterol stearate, 10 = triolein).

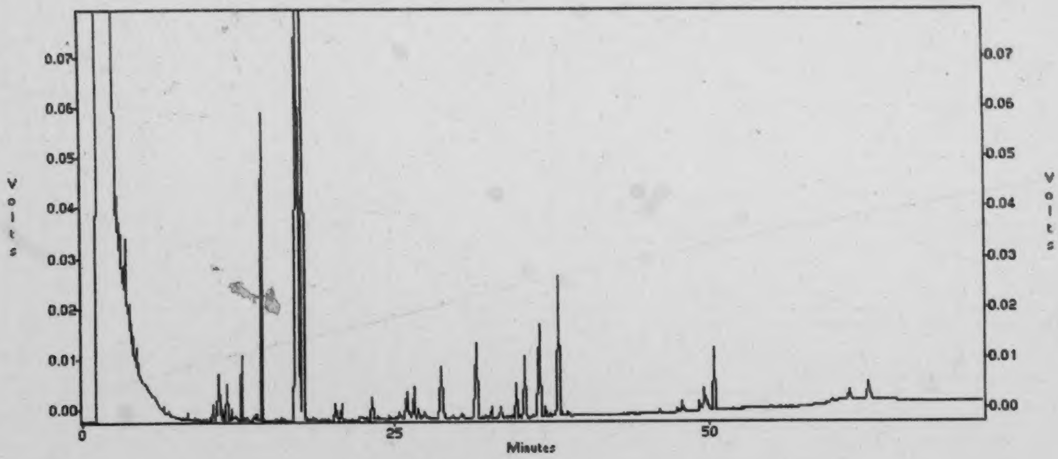

Figure 6.3. A typical GC chromatogram of the derivatized sample.

Table 6.2. Composition of the canola oil deodorizer distillate.

Component	Concentration (%)[a-c]
Tocopherols[a]	
α-	1.44±0.08
γ-	3.88±0.20
δ-	2.78±0.15
Total	8.11±0.43
Sterols[a]	
brassicasterol	2.86±0.15
campesterol	4.69±0.25
stigmasterol	0.37±0.02
sitosterol	6.54±0.34
Total	14.73±0.38
Free fatty acids[b]	50.39±1.13
Fatty acids[c]	
C16	13.97±0.16
C18	22.29±0.15
C18:1	52.73±0.37
C18:2	7.17±0.06
C18:3	2.46±0.04
C20	0.85±0.06
C20:1	0.53±0.01

[a] % w/w
[b] % as oleic acid
[c] GC Area %

6.2.4.3. Quantitative analysis

The composition of the feed, fractions and residue were reported as GC area %. Quantification of sterols and tocopherols in the feed was carried out using internal standard (IS) method. A standard solution containing known amounts of oleic acid, monoolein, diolein, cholesterol stearate, triolein, α- and δ-tocopherols (95 and 90% pure, respectively) and stigmasterol (95% pure), and C13 triglyceride (tritridecanoin, Nu-Chek-Prep Inc., Elysian, MN) as an internal standard was analyzed and relative response factors (RRF) were calculated for sterols and tocopherols using the formula:

$$\text{RRF} = \frac{\text{weight of standard X area of IS}}{\text{weight of IS X area of standard}} \qquad (6.1)$$

Stigmasterol standard was used to calculate the RRFs for the sterols, whereas RRFs were calculated for both α- and δ-tocopherol and an average of these RRFs were used for the remaining tocopherols. RRFs were determined to be 0.80 (±0.002) for α-tocopherol, 0.83 (±0.057) for δ-tocopherol and 0.86 (±0.035) for stigmasterol. A known amount of IS (0.4 mL) was also added to the sample to be analyzed and the weight of components of interest in the sample was calculated as

$$\text{Weight}_x = (\text{RRF}_x \text{ X weight of IS X area}_x) / \text{area of IS} \tag{6.2}$$

6.2.5. Statistical analysis

All fractionation experiments were replicated twice. GC analysis of each fraction obtained in each run was carried out in duplicate. Analysis of variance (ANOVA) was carried out using General Linear Model Procedure of SAS Statistical Software [17] to test the effect of temperature and pressure on the total fractionation yield and residue composition. Multiple comparison of the means were carried out using pdiff option of the LSMEANS (Least Squares Means) statement at p=0.05. An adjusted ANOVA for repeated measurements was also performed to test the effect of time on fractionation yield and fraction composition.

6.3. RESULTS AND DISCUSSION

Canola oil deodorizer distillate was fractionated using a $SCCO_2$ fractionation column to test the performance of the column and to assess the feasibility of the column fractionation for the value-added processing of this particular feed material. In addition, the effects of temperature and pressure on the yield and composition of fractions obtained were investigated. Physical and chemical properties of mixture components and the effect of operating conditions on yield and selectivity of the fractionation process were considered while choosing the experimental conditions to be tested. The solubility behavior of lipid components in $SCCO_2$ and the implications of the findings for processing have been discussed in detail in Chapters 2-5. Pressure will affect the feasibility of the process through its effect on selectivity and yield. Yield of the extract will increase, whereas that of raffinate will decrease with increasing pressure. In general, selectivity of the separation decreases with pressure; however, it may also increase with pressure. The net pressure effect is determined by mixture composition and targeted separation as discussed in Section 6.3.3. The lower operating pressure was chosen as 20 MPa, considering the yield/solvent loading and its effects on residue composition as well as fractionation time. The effect of yield on residue composition is determined by the amount of feed introduced into the column, whereas yield at a given time is also affected by column volume and CO_2 flow rate. Highest pressure was chosen as 25 MPa, as lower selectivity was expected at higher pressures for the targeted separation (also based on information on multicomponent solubility data of similar deodorizer distillate mixtures). Lowest temperature limit was chosen to ensure that the feed will be in liquid phase during fractionation experiments to avoid any potential plugging of the column upon its solidification. The higher temperature limit was set considering the heat sensitivity of mixture components and to avoid any degradation.

Composition of the feed material and the fractions obtained under investigated conditions are reported in Tables 6.2 and 6.3, respectively. Sterol and tocopherol contents of the canola oil deodorizer distillate used in this study were higher than those reported by Ramamurthi et al. [8]. This is an expected result because of the inherent variability in

Table 6.3. Composition of feed, fractions and residues of $SCCO_2$ column fractionation of canola oil deodorizer distillate.

	GC Area%														
	Volatiles	FFA	MG	Sq	Tocopherols				Sterols					DG	TG
					δ-	γ-	α-	Total	Brassica-	Campe-	Stigma-	Sito-	Total		
Feed	7.10	61.52	4.78	0.43	2.27	3.18	1.21	6.66	2.29	3.77	0.31	5.16	11.53	2.42	1.86
25MPa @ 70°C															
F1	33.08	50.62	3.53	0.55	2.00	1.21	0.62	3.82	0.72	1.05	0.08	1.35	3.20	0.43	0.24
F2	20.69	61.84	4.45	0.62	2.43	1.56	0.77	4.76	0.77	1.11	0.09	1.39	3.36	0.46	0.14
F3	10.77	68.94	5.00	0.59	2.55	1.89	0.95	5.39	0.95	1.38	0.12	1.77	4.21	0.76	0.28
F4	5.60	72.62	5.08	0.47	2.57	2.37	1.15	6.08	1.26	1.86	0.16	2.32	5.60	1.04	0.29
F5	3.18	71.08	5.35	0.37	2.24	3.01	1.37	6.62	1.85	2.67	0.23	3.46	8.21	1.56	0.36
Residue	5.14	61.10	4.52	0.38	2.87	3.22	1.25	7.34	2.59	4.26	0.34	5.92	13.11	2.80	2.00
25MPa @ 70-100°C															
F1	29.06	51.47	4.38	0.62	2.40	1.45	0.74	4.59	1.04	1.64	0.11	2.18	4.97	0.59	0.16
F2	17.52	63.66	5.78	0.74	2.98	1.58	0.83	5.39	0.73	1.01	0.07	1.27	3.08	0.25	0.01
F3	8.52	72.64	5.44	0.61	3.40	1.83	0.97	6.21	0.75	0.97	0.07	1.16	2.95	0.32	0.13
F4	3.60	76.41	5.45	0.51	3.52	2.23	1.16	6.91	0.87	1.11	0.09	1.36	3.43	0.39	0.09
F5	1.70	75.21	6.43	0.42	2.44	2.92	1.46	6.82	1.33	1.70	0.14	2.00	5.18	0.51	0.01
Residue	1.89	29.89	2.58	0.15	1.20	3.28	0.91	5.39	7.12	12.73	0.99	19.56	40.41	5.24	9.16
25MPa @ 100°C															
F1	33.97	45.83	3.41	0.58	2.84	1.39	0.65	4.88	1.08	1.99	0.12	2.52	5.71	0.58	0.95
F2	23.76	57.40	5.08	0.83	3.36	1.73	0.85	5.93	0.72	1.01	0.08	1.26	3.06	0.31	0.22
F3	13.92	65.43	5.79	0.87	4.10	1.94	0.99	7.03	0.74	1.03	0.08	1.28	3.13	0.22	0.04
F4	8.19	70.30	5.94	0.72	3.68	2.22	1.17	7.08	0.86	1.17	0.08	1.45	3.56	0.27	0.08
F5	5.84	70.17	6.27	0.55	3.22	2.62	1.42	7.26	1.17	1.54	0.12	1.92	4.75	0.70	0.42
Residue	8.55	52.15	5.03	0.51	3.08	3.07	1.34	7.49	2.56	4.20	0.33	5.94	13.04	3.16	3.24
20 MPa @ 70-100°C															
F1	44.74	40.04	3.43	0.72	2.27	1.03	0.54	3.84	0.61	0.95	0.05	1.22	2.82	0.37	0.31
F2	37.98	47.21	4.65	0.92	3.45	0.88	0.48	4.81	0.23	0.24	0.00	0.30	0.77	0.00	0.05

Table 6.3. continued

	GC Area%														
	Volatiles	FFA	MG	Sq	Tocopherols				Sterols					DG	TG
					δ-	γ-	α-	Total	Brassica-	Campe-	Stigma-	Sito-	Total		
F3	26.58	56.08	6.13	1.18	3.67	1.11	0.60	5.38	0.24	0.23	0.00	0.27	0.74	0.00	0.05
F4	16.58	63.44	6.76	1.31	4.51	1.31	0.75	6.57	0.26	0.26	0.00	0.29	0.81	0.00	0.15
F5	11.22	68.79	7.03	1.15	5.17	1.45	0.81	7.42	0.27	0.24	0.00	0.29	0.80	0.00	0.09
Residue	2.59	65.41	5.34	0.31	2.87	2.71	1.07	6.66	2.42	3.84	0.31	5.32	11.89	2.11	1.94

oil composition and the dependence of distillate composition on deodorization and storage conditions [2].

6.3.1. Fractionation yield

Fractionation yield is defined as the cumulative weight of the fractions at a certain time during the fractionation. It is determined by loading of the solvent and thus the solubility behavior under the investigated conditions. Total fractionation yield increased significantly ($p \leq 0.05$) with pressure (Fig. 6.4, Table 6.4). An increase in yield with pressure is expected due to an increase in the solvent power of $SCCO_2$ and has been reported in both phase equilibrium and fractionation studies of multicomponent lipid mixtures such as deodorizer distillates [3-7, 18-20] and vegetable oils [21, 22].

At isobaric conditions (25 MPa), the highest yield was obtained at the lowest temperature tested (70 °C), whereas the yield obtained using a temperature gradient was between those obtained under isothermal conditions of 70 °C and 100 °C; however, these differences were not significant ($p>0.05$) (Fig. 6.4, Table 6.4).

Temperature dependence of fractionation yield is determined by the overall effect of temperature on the solubility behavior of mixture components and is thus affected by mixture composition. Decreasing fractionation yields with increasing temperature have been reported for esterified olive oil deodorizer distillates (at 11-15 MPa and 40-60 °C) [21] and rice bran deodorizer distillate (at 20-32 MPa and 45-80 °C) [22]. A similar temperature effect had been reported for the pseudobinary solubility behavior of deacidified palm oil enriched with sterols or carotene (26 MPa, 50-100 °C) and palm oil deodorizer distillate [19]. Pseudobinary solubility of soybean oil deodorizer distillate showed a minimum with temperature at 90 °C or decreased with temperature for different

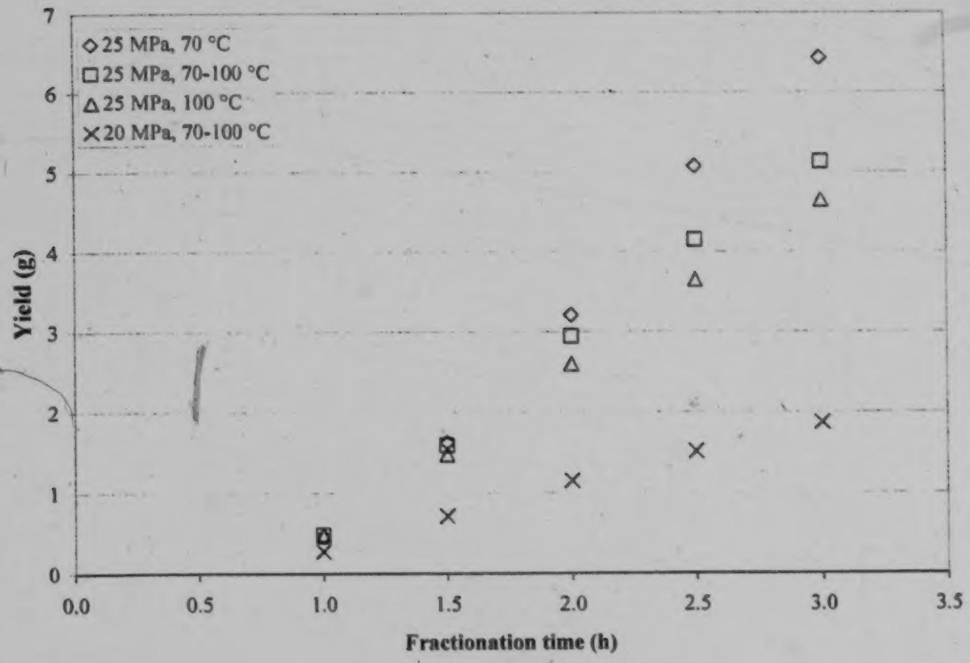

Figure 6.4. Fractionation yields of $SCCO_2$ column fractionation of canola oil deodorizer distillate.

Table 6.4. Total fractionation yield and yield of collected fractions of $SCCO_2$ column fractionation of canola oil deodorizer distillate.

	Temperature effect at 25 MPa		
	Yield (g)		
	70 °C	**100 °C**	**70-100 °C**
Fraction 1	0.47	0.47	0.48
Fraction 2	1.16	1.01	1.11
Fraction 3	1.59	1.12	1.35
Fraction 4	1.86	1.05	1.20
Fraction 5	1.36	0.99	0.98
Total [1]	6.43±1.6[a]	4.64±0.06[a]	5.13±0.49[a]

	Pressure effect at 70-100 °C	
	Yield (g)	
	20 MPa	**25 MPa**
Fraction 1	0.277	0.48
Fraction 2	0.436	1.11
Fraction 3	0.435	1.35
Fraction 4	0.366	1.20
Fraction 5	0.356	0.98
Total[1]	1.87±0.05[a]	5.13±0.49[b]

[1] Mean ± standard deviation

[ab] Means in the same row with the same superscript are not statistically different ($p>0.05$).

tocopherol concentrations [20]. While pseudobinary solubility data of sterol removed soybean distillate pointed to a crossover of isotherms at approximately 30 MPa, which indicates persistence of crossover behavior in multicomponent lipid systems, esterified distillate solubility decreased with temperature in the range of 35-70 °C at 20-30 MPa [23].

Similar to the temperature and pressure effects on total fractionation yield, average yield of the fractions were similar for all temperatures ($p>0.05$); however, a significant difference was observed due to pressure effect. Fraction yields varied with fractionation time/fraction number with significant linear and parabolic trends (reflected as a maximum in the fraction yield curves, Fig. 6.5), which was significantly different

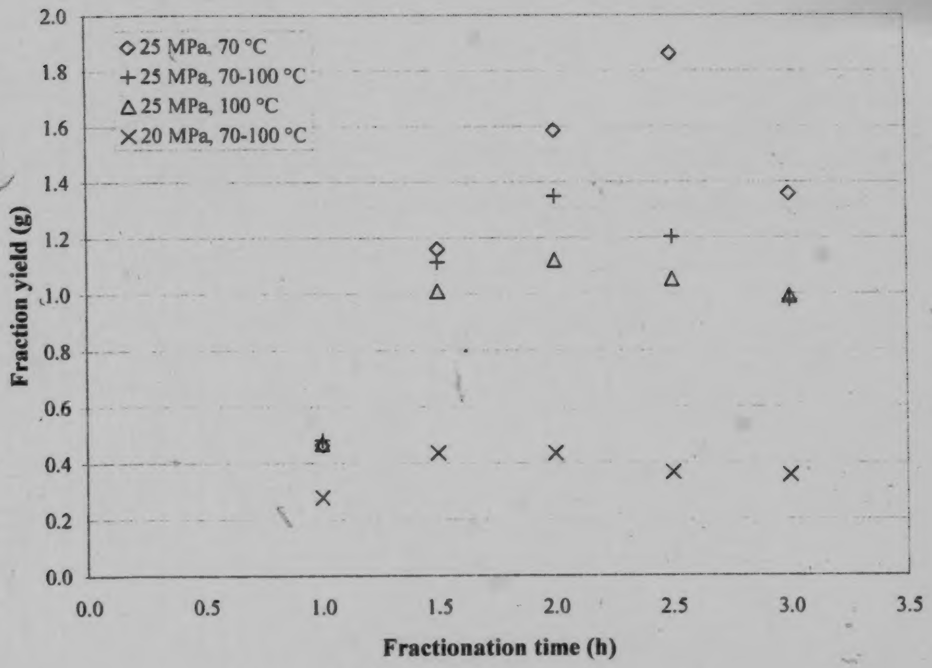

Figure 6.5. Yield of fractions of SCCO$_2$ column fractionation of canola oil deodorizer distillate.

($p \leq 0.05$) between the pressures but not temperatures. Time dependence of fraction yields was similar for all temperatures; however, differed between pressures. The decrease in fraction yield with time reflects the changing composition of the distillate due to the batch nature of the processing protocol applied and occurs due to the depletion of the more soluble components of the mixture.

6.3.2. Composition of fractions

At every condition tested, volatiles content of the fractions decreased whereas that of FFA, tocopherols and sterols increased with fractionation time/fraction number (Figs. 6.6-6.9). Statistical analysis of the data revealed that the average volatile, FFA and tocopherol contents of the fractions were significantly ($p \leq 0.05$) affected by temperature and pressure, whereas only pressure had a significant ($p \leq 0.05$) effect on sterol content.

Increasing the fractionation pressure increased the content of all fraction components except volatiles, which points to decreasing selectivity of the separation of volatiles from the rest of the mixture components. Temperature effect varied between components, while for tocopherols it followed the order 100 °C> 70-100 °C>70 °C; for sterols the order was reversed after 1.5 h. The effect on FFA were 70-100 °C>70 °C>100 °C, whereas for volatiles 100 °C> 70 °C>70-100 °C.

The change in the volatiles and FFA contents of the fractions with fractionation time was significant ($p \leq 0.05$) and differed between pressures (but not temperature). There was also a significant time effect on sterol and tocopherol contents, which differed significantly ($p \leq 0.05$) between temperatures for sterols and between pressures for tocopherols.

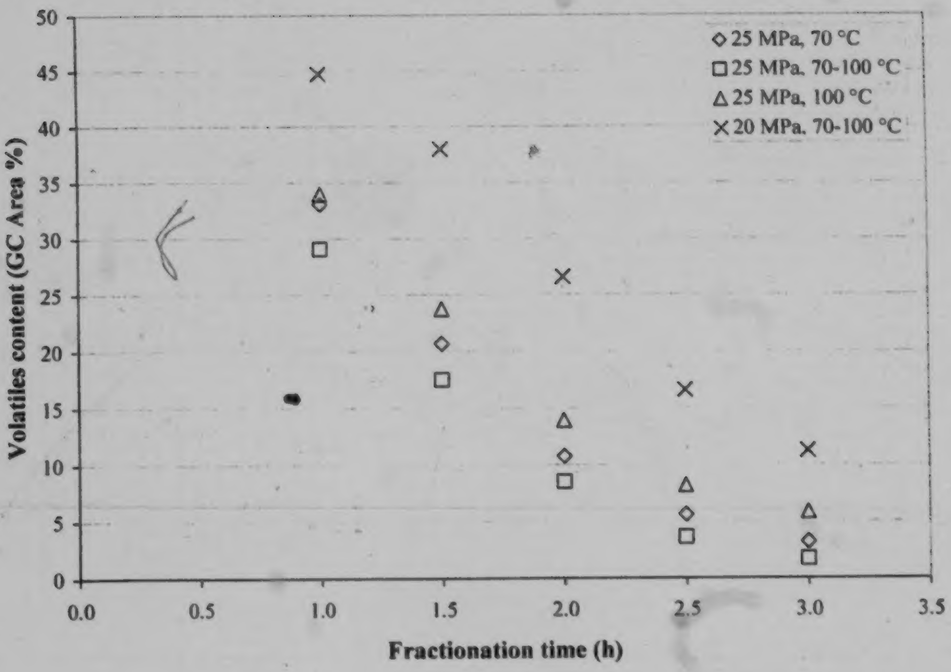

Figure 6.6. Volatile content of the fractions of $SCCO_2$ column fractionation of canola oil deodorizer distillate.

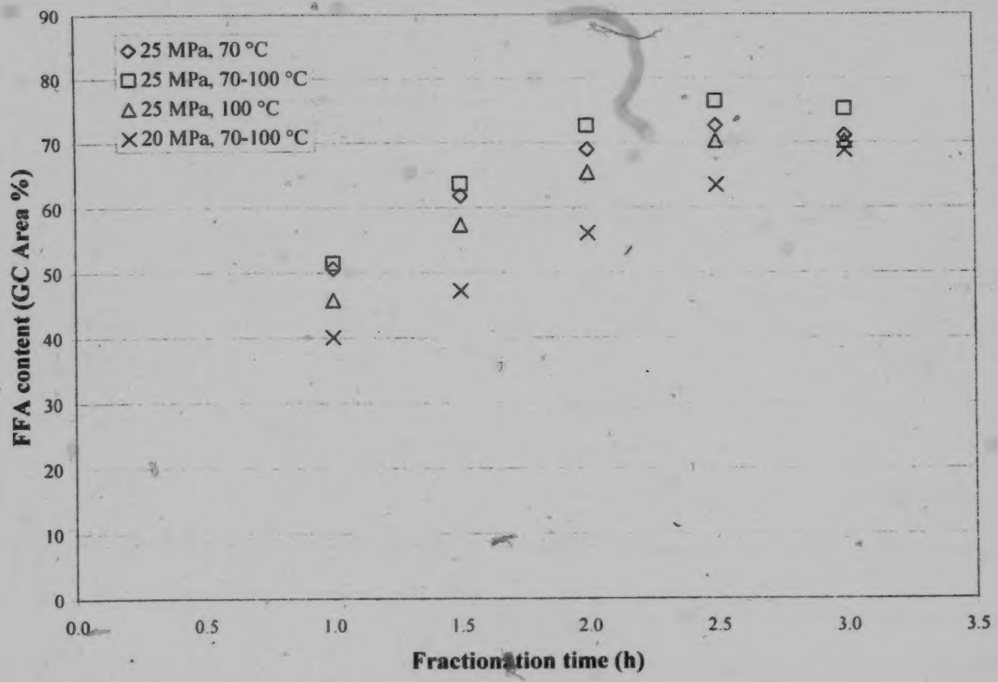

Figure 6.7. FFA content of the fractions of $SCCO_2$ column fractionation of canola oil deodorizer distillate.

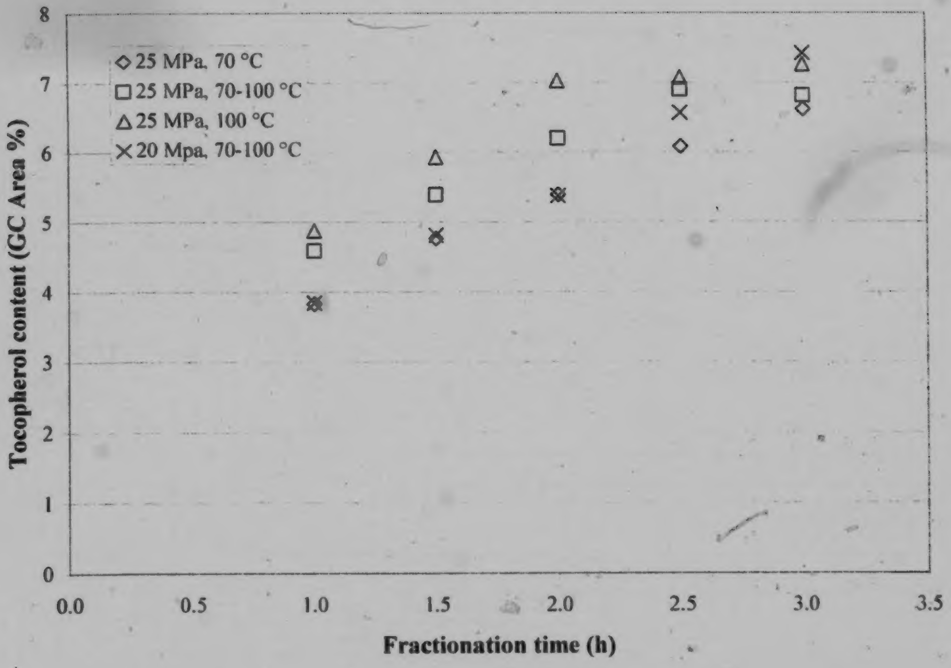

Figure 6.8. Total tocopherol content of the fractions of $SCCO_2$ column fractionation of canola oil deodorizer distillate.

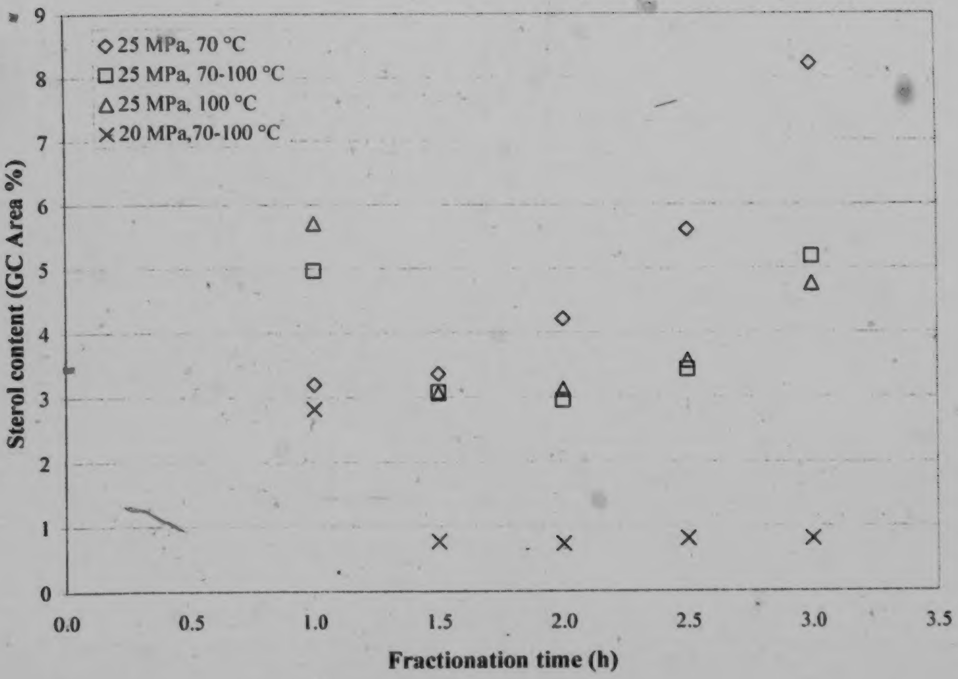

Figure 6.9. Total sterol content of the fractions of $SCCO_2$ column fractionation of canola oil deodorizer distillate.

Analysis of trends with temperature as a factor yielded significant linear and parabolic trends for the volatile, FFA and tocopherols, and a significant parabolic trend for sterols, neither of which interacted with temperature. When pressure data were analyzed, in addition to the linear and parabolic trends a cubic trend was observed for volatile content, which varied with pressure. FFA showed significant linear and parabolic trends, which were affected by pressure. A significant linear and parabolic trend was observed for tocopherol and sterol content, respectively, neither of which was affected by pressure.

The distribution of mixture components between raffinate and extract streams will be affected by the specifics of the fractionation process, operating conditions and sample composition. Enrichment of mixture components can be predicted to a certain extent by their relative binary solubility; however, for components with similar solubilities such as tocopherols and FA, or sterols and TG deviations will occur, as the differences in the effects of operating conditions on solubility and the effect of the presence of other solutes would be reflected in the relative solubilities of mixture components.

Phase equilibrium measurements of soybean oil deodorizer distillate were reported to show enrichment for high volatile components (including FFA), squalene and medium volatile components in the vapor phase, whereas sterols and tocopherols were enriched in the liquid phase [18, 20]. SFE of soybean distillates yielded enrichment of FFA in the extract, whereas sterols and tocopherols were enriched in the residue [23, 24]. Chang et al. [25] however reported tocopherol enrichment in the extract in their study on fractional extraction of tocopherols using a reboiler to create a thermal gradient. During column fractionation of soybean distillates, tocopherol enrichment in both extract and raffinate phases has been reported [3, 6].

The dependence of separation behavior on mixture composition was observed for palm oil deodorizer distillate [19], where distribution coefficient of linoleic acid increased (reversing its enrichment behavior) with increasing concentration of tocopherols in the mixture.

The higher binary solubility of stigmasterol than triolein observed at 323 K (Chapter 3) was not reflected in the multicomponent phase behavior of palm oil enriched with sterols, where enrichment of sterols was observed in the vapor and triolein in the liquid phase [20]. This deviation can be attributed to the different temperature dependence of these components; furthermore, the presence of triolein may have a diluting effect and enhance the solubility of sterols in the multicomponent mixture.

Temperature had a significant effect on the composition of the residues obtained at different conditions (Table 6.5, Fig. 6.10). Increasing the temperature from 70 to 100 °C increased the volatile content significantly ($p \leq 0.05$), whereas FFA, tocopherol and sterol contents were not affected. Use of the thermal gradient decreased the content of volatiles, FFA and tocopherols although the decrease in volatiles was not significant ($p > 0.05$). On the other hand, sterol content increased significantly ($p \leq 0.05$) when temperature gradient was applied, reaching a level of 40%. Pressure did not have a significant effect on volatiles, while increasing the sterol and decreasing the tocopherol and FFA contents. It should be noted that the reported compositions are for the residue collected upon depressurization of the column (0.04-3.45 g) and does not represent the composition of the remaining raffinate. At the end of each run, the raffinate is expected to

Table 6.5. Composition of residues of $SCCO_2$ column fractionation of canola oil deodorizer distillate.

	Residue composition (GC Area%)			
	volatiles	FFA	tocopherols	sterols
25 MPa		**Temperature effect**		
70 °C	5.14^{a}	61.10^{a}	7.34^{a}	13.11^{a}
70-100 °C	1.89^{a}	29.89^{b}	5.39^{b}	40.41^{b}
100 °C	8.55^{b}	52.15^{a}	7.49^{a}	13.04^{a}
70-100 °C		**Pressure effect**		
20 MPa	2.59^{x}	65.41^{x}	6.66^{x}	11.89^{x}
25 MPa	1.89^{x}	29.89^{y}	5.39^{y}	40.41^{y}

^{ab} Means in the same column with different superscripts are statistically different ($p \leq 0.05$).
^{xy} Means in the same column with different superscripts are statistically different ($p \leq 0.05$).

be distributed along the column height with a concentration gradient. The reported residue compositions are indicative of the separation efficiency for the studied mixture.

6.3.3. Fractionation efficiency

Fractionation efficiency is reflected in the composition of the fractions (Table 6.3); however, its effect on the composition of the residue can be masked by the low fractionation yield (as observed at 20 MPa). For example at 20 MPa, although sterols were not extracted to any appreciable extent, no enrichment could be achieved in the raffinate (Table 6.3) as the extracted amounts of other components were substantially lower than the amount of raffinate and hence did not affect the composition of the raffinate.

In a fractionation process, solvent power and selectivity are two parameters that affect the efficiency of a process. Usually, in $SCCO_2$ fractionation processes these two parameters show opposing trends, which has been noted by various researchers such that selectivity decreases with increasing solvent power, and hence with pressure [3, 6]. This behavior has been generally accepted as a norm and offers a processing challenge as high

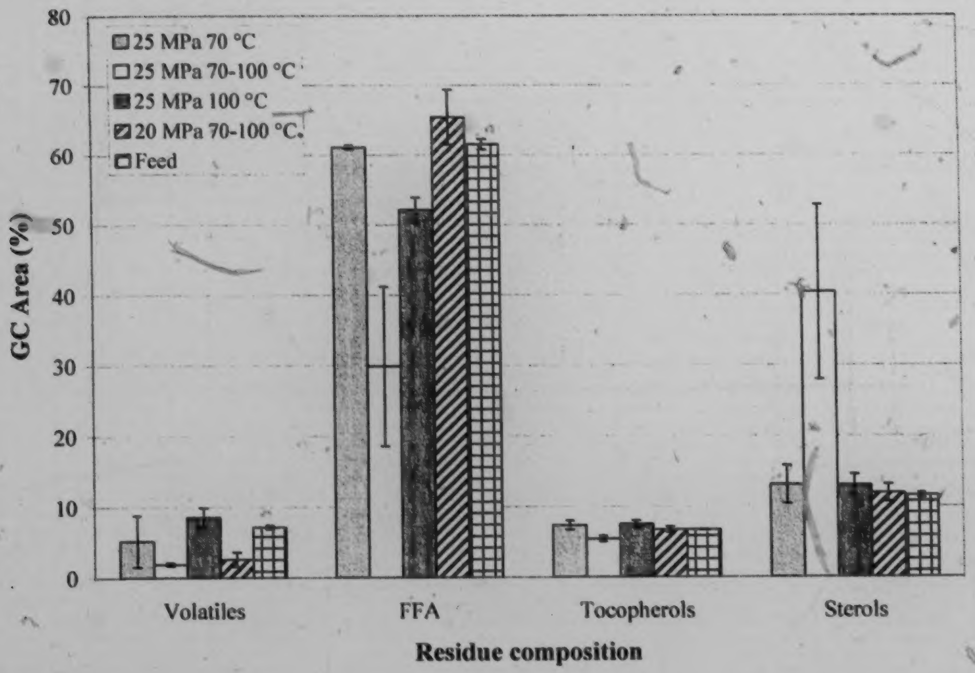

Figure 6.10. Composition of residues of $SCCO_2$ column fractionation of canola oil deodorizer distillate.

solvent power and selectivity values are required for an efficient process. An increase in selectivity values with pressure hence solvent power has also been reported although for a small number of systems such as palm oil deodorizer distillate (tocopherols/FFA) [19]. Therefore, no assumptions/generalizations should be made. It should also be noted that the pressure effect may also differ for different separations targeted in the same system.

6.4. CONCLUSIONS

Semi-continuous $SCCO_2$ column fractionation of canola oil deodorizer distillate was carried out to determine the feasibility of value-added processing of this feed material and the effect of operating conditions on fractionation yield and efficiency noted.

Although canola oil deodorizer distillate has previously been regarded as a FFA source, the results of this study using $SCCO_2$ technology show the potential of this raw material as a sterol source. Semi-continuous processing of the distillate at 25 MPa and 70-100 °C yielded a residue containing 40% sterols. It should be noted that the efficiency of this separation can be further improved by changes in the process design such as continuous processing of the distillate and the use of a reflux pump to generate an external reflux.

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7. CONCLUSIONS AND RECOMMENDATIONS

Supercritical CO_2 ($SCCO_2$) fractionation of fats and oils has been widely investigated since $SCCO_2$ offers an environmentally friendly alternative for the processing of fats and oils with added advantages such as moderate operating conditions and solvent-free extracts and residues.

From a processing perspective, a unique advantage of $SCCO_2$ processing lies in its versatility, which results from the ability to modify solvent properties by changing operating conditions (temperature and pressure) or by the addition of cosolvents. This operational flexibility enables the processor to fine tune solvent properties for the specific separation problem at hand.

A good understanding of the fundamentals of solubility behavior of lipid components in $SCCO_2$ as affected by operating conditions and solute properties is required to realize its full potential in fats and oils processing. Predictive modeling of solubility behavior in supercritical fluids has not been realized yet; therefore, experimental solubility data play a key role in the study of solubility behavior. The accuracy/reliability of the experimental data also determines the success of thermodynamic modeling studies. Therefore, in this thesis research the solubility behavior of lipid components has been analyzed using a systematic approach from simpler binary to multicomponent mixtures to determine the general trends of solubility behavior as affected by temperature, pressure and mixture composition, to study the deviation from binary behavior as affected by feed composition and to assess the implications for fractionation processes. In addition, fractionation of a complex lipid mixture, canola oil deodorizer distillate, was carried out using a $SCCO_2$ fractionation column to achieve value-added processing of this by-product of conventional canola oil refining process.

7.1. SOLUBILITY BEHAVIOR OF LIPIDS AND IMPLICATIONS ON PROCESSING OF FATS AND OILS

General trends of solubility behavior were established by studying the available literature solubility data of binary systems of lipids and $SCCO_2$ (Chapters 2 and 3). The physical state of the solutes was found to have a major effect on the observed solubility trends. In general, solid solutes (such as stigmasterol, β-carotene and solid fatty acids) had a low crossover pressure and therefore showed retrograde behavior in the practical processing range. Although the presence of a crossover point in solid systems has been well established previously, liquid systems and the effect of melting on the crossover behavior have not been addressed adequately. While a crossover pressure was observed for some liquid lipid components, solubility of liquid solutes such as fatty acid esters decreased with temperature in the investigated experimental range. Solubility increased with pressure for all the studied solutes, whereas a solubility maximum was observed in the solubility isotherms of solid solutes such as stearic acid, β-carotene and β-sitosterol at high pressures. Although investigated liquid systems did not exhibit similar behavior, solubility maxima have been well established for vegetable oils at high pressures.

Solubility behavior in $SCCO_2$ was determined by vapor pressure and intermolecular interactions and hence was affected by operating conditions and solute properties. In binary systems of a homologous series, where intermolecular interactions were similar, solubility was governed by molecular weight/vapor pressure, whereas unsaturation affected solubility mainly through its effect on the physical state of the solute. In an analogous series, the relative effect of vapor pressure and intermolecular interactions was dependent on solvent density as observed in olein and laurin glyceride series such that intermolecular interactions hence polarity was the dominant factor at high densities. While α-tocopherol, liquid fatty acids (oleic acid) and squalene were the most soluble one's amongst the studied solutes; β-carotene had the lowest solubility in $SCCO_2$.

Although the physical state of the solute, thus melting had a significant impact on lipid solubility behavior, information on melting behavior of lipids in $SCCO_2$ was rather limited. Further research is required to provide information on the melting behavior of lipids in binary and multicomponent systems. Melting behavior of solutes should be noted during solubility measurements directly (using a view cell) or indirectly by studying the solute after the measurements for any evidence of melting. Physical state of the solutes should always be considered while using binary data to compare the solubility behavior of different solutes to avoid reaching wrong conclusions.

The study of the solubility database revealed wide discrepancies between experimental data reported by different researchers, possible sources of which were identified as the limitations of the experimental methods used and purity of samples. Although discrepancies were observed for a number of solutes, it had a significant effect on the solubility behavior of a sensitive solute of low solubility such as β-carotene. Impurities in the samples arising from sample degradation and isomeric purity of the samples contributed to this variation. This finding highlights the need to exercise extreme caution not only while making solubility measurements but also while interpreting the data. Measures should be taken to limit sample degradation during handling, processing, analysis and storage.

Binary data can be used to determine the optimum fractionation conditions in the design of fractionation processes for mixtures such as fatty acid esters. For more complex mixtures containing different lipid classes such as deodorizer distillates or for mixtures/mixture components, which undergo melting the effect of operating conditions on multicomponent solubility behavior can vary greatly dependent on mixture composition. Regardless, binary solubility data can still provide valuable information on the fractionation of lipid components and can be used to assess the potential of the required separation. For example, fractionation of solutes with similar binary solubilities such as fatty acids, tocopherols and squalene would be very difficult to achieve and may warrant additional processing steps, whereas solutes with different solubilities such as triglycerides/sterols and fatty acids can be separated with ease in a fractionation column. Binary data can thus be used as a basis for the study of multicomponent mixtures; however, limitations of their predictive ability should not be overlooked.

Solubility behavior of lipids in ternary systems containing lipid + cosolvent + CO_2 were studied to interpret and correlate the cosolvent effect for lipid components (Chapter 4). The effects of cosolvent addition on various aspects of $SCCO_2$ processing were also assessed. The addition of a small amount of a cosolvent modified the solvent power of $SCCO_2$ by enhancing solute solubility. The observed solubility enhancements were attributed to an increase in solvent density and intermolecular interactions. The highest solubility enhancement observed in ethanol and fatty acid systems were attributed to H-bonding interactions. Such specific intermolecular interactions between a solute and a cosolvent can be exploited for fractionation of lipid mixtures. In the literature, the advantages of cosolvent addition have been emphasized; however, the effects of cosolvents on different aspects of the process such as mass transfer, overall cost and product/residue properties have not been considered. Benefits of cosolvent addition must be balanced against its disadvantages for a specific application. Cosolvent introduction and solvent recovery (separation of the cosolvent from the extract, supercritical fluid and solids residue) increase the complexity of process design. An increase in solvent loading may result in the co-extraction of undesirable compounds. The effect of cosolvent addition on the sample matrix and solutes of interest such as alteration of functional properties of extraction residue and degradation of the extract by the cosolvent should also be considered. Use of cosolvents would override one of the major advantages offered by $SCCO_2$, namely the ability to produce "natural" products with no organic solvent residue. Ethanol is the cosolvent of choice in food applications due to its GRAS status, but the removal of ethanol from the extract and residue requires the application of heat, which may be detrimental to the quality of the extract and residue. This step also undermines another advantage of $SCCO_2$ extraction that it can be carried out at just above ambient temperatures.

Predictive ability of binary data was limited in ternary and higher lipid systems as multicomponent solubility deviated from binary behavior (Chapter 5). While solubility of the less soluble component increased in ternary systems of solid triglycerides in $SCCO_2$, that of the more soluble component decreased or was not affected. Solubility diminution was observed for both solutes in some liquid mixtures such as fatty acid (oleic acid/linoleic acid) and fatty acid ester (methyl oleate/methyl linoleate) mixtures. The extent of this diminution was dependent on the initial feed concentration of the solute. Solubility enhancement for one of the mixture components was also observed (for example for oleic acid in the presence of methyl oleate). These deviations in turn affected the separation efficiency (assessed in terms of partition coefficient and selectivity) of the solutes of interest. Separation efficiency was lower than that predicted by binary data when the solubility of the less soluble solute was enhanced in the mixture, whereas it was improved if the solubility of the more soluble component was enhanced as observed in quaternary glyceride mixtures. The presence of a less polar (diluting) component in the mixture led to solubility enhancement of a polar mixture component as observed for monoolein and diolein in the presence of triolein. This dilution effect can have important implications for fractionation processes as it may improve the separation efficiency significantly if the more polar components are more soluble than the diluting component. High selectivity values obtained for oleic acid in oleic acid/methyl oleate and oleic acid/linoleic acid systems require further attention for confirmation and determination of their practical significance.

The ultimate goal in the study of solubility behavior is to achieve predictive modeling of solubility data such that the experimental workload required for process design is minimized. The comprehensive and critical analysis carried out in this thesis research provides valuable insight into the available lipid solubility data and outlines the effects of solute properties and operating conditions on solubility behavior of numerous lipid components. It also establishes the general trends of the deviation observed in multicomponent systems with a focus on solute properties (intermolecular interactions present in the system). This state of knowledge now provides a sound basis for further in-depth thermodynamic modeling studies, which can target predictive capability. The approach used in this study for ternary and higher systems can be extended to study the multicomponent solubility behavior and the nature and extent of the deviations from binary behavior observed in multicomponent systems containing lipid mixtures such as deodorizer distillates. Although Chrastil's model provided an effective tool to meet the objectives of this thesis research, the same properties that made it the correlation of choice in this study, its simplicity and empirical nature, in effect limits its use in the study of modeling of solubility data especially of multicomponent mixtures as it does not take intermolecular interactions into account. Further research in modeling of lipid solubility data hence should involve in-depth thermodynamic modeling.

Physicochemical properties of the lipids and solvents and cosolvents were used to interpret the observed deviations from binary behavior observed in multicomponent systems, whenever such data were available. Physicochemical properties of studied lipid components and cosolvents were compiled in this thesis research; however, the database of lipids is not complete. A systematic study of the properties of lipid components would be of great value. Intermolecular interactions play a significant role in determining the solubility behavior of solutes especially in multicomponent systems. Although intermolecular interactions in supercritical systems have been studied for various solutes in CO_2 and CO_2 + cosolvent systems, the data on lipids are rather scarce. Study of intermolecular interactions in lipid systems will further our understanding of solubility behavior and hence aid in modeling.

A good understanding of the physical and chemical properties of solutes of interest is also an integral part of the processing of natural materials. Knowledge of solute properties is required not only to minimize degradation of solutes during handling, processing and storage but also for accurate interpretation of solubility data.

The implications of the findings of the study of solubility behavior of lipids in $SCCO_2$ were assessed for processing of fats and oils for extraction and fractionation purposes to provide a processing framework (Chapters 2-5). The use of solubility behavior information for the design of extraction and fractionation processes and its relevance to process performance were identified.

7.2. $SCCO_2$ COLUMN FRACTIONATION

7.2.1. $SCCO_2$ fractionation column

A fractionation column was designed and built to carry out $SCCO_2$ column fractionation. The column was designed for maximum operational flexibility. The location of CO_2 and feed introductions can be varied along the column through cross/tee fittings, which can also potentially be used to achieve sampling from various points along the column. Further modifications in column design should aim to minimize experimental variation and process time and to improve separation efficiency. Introduction of the melted deodorizer distillate into the column without solidification was one of the major processing challenges encountered. It was not possible to pump the feed material without solidification in the pump using the available HPLC-type feed pumps despite heating of the feed lines and the pump components. Therefore, a peristaltic pump was used for feed introduction. A new feed pump that would enable the introduction of melted feed material without solidification would simplify the process and decrease the processing time. A micrometering valve is needed on the raffinate line for efficient depressurization of the system. Multiple thermocouples placed inside the column along the column height to monitor the temperature differential will provide a better picture of the temperature profile of the fluid inside the column.

The next phase in the $SCCO_2$ column fractionation research should focus on continuous countercurrent operation of the column to improve the separation efficiency. The requirements for countercurrent processing include a high-pressure feed pump, operating at flow rates compatible with CO_2 flow rates. In addition to phase equilibria, design of a countercurrent process should also involve the investigation of mass transfer aspects (determination of flooding and pressure drop, determination of height equivalent to a theoretical plate (HETP)) [1]. Therefore, future research should focus on mass transfer characteristics of the column. A view cell would enable the observation of the liquid level in the column. Separation efficiency can be further improved by an external reflux, which will require an additional high pressure feed pump.

7.2.2. $SCCO_2$ column fractionation of canola oil deodorizer distillate

Canola oil deodorizer distillate has previously been considered as a source of fatty acids due to its high fatty acid and low sterol/tocopherol content [2]. Results of this thesis research indicate its potential as a source of sterols, which are receiving increasing attention due to their health benefits. Concentration of sterols up to 40% (GC Area %) was achieved in the residue of the fractionation process at 25 MPa using a thermal gradient along the column (70-100 °C).

Further improvements in separation efficiency can be achieved using different fractionation approaches. For components that are difficult to separate due to similar solubilities in $SCCO_2$ such as squalene and fatty acids or fatty acids and tocopherols, modification of the feed material composition (esterification of the free fatty acids with lower alcohols to convert them into highly soluble methyl/ethyl esters) can be used as a pretreatment step to increase the efficiency of the separation by increasing the solubility gradient between mixture components. This approach has been successfully used for the concentration of squalene from olive oil deodorizer distillate [3], where free fatty acids and fatty acid esters were converted into triglycerides to produce a highly enriched squalene fraction. Esterification of free fatty acids in deodorizer distillates has also been investigated to achieve the concentration of sterols and tocopherols [4, 5]. This approach could improve the separation efficiency of free fatty acids and sterols considerably due to large solubility gradient between sterols and fatty acid esters, which are at the opposite ends of the solubility spectrum. An interesting possibility will be to study this esterification reaction in $SCCO_2$. Another approach includes the use of a pressure gradient in addition to a thermal gradient, such that an increasing pressure gradient is applied throughout the fractionation, enabling the removal of more soluble components in the initial stages of the fractionation.

7.3. COMMERCIALIZATION OF SUPERCRITICAL FLUID TECHNOLOGY AND IMPLICATIONS FOR FUTURE RESEARCH

Although the use of supercritical fluid solvents offers a flexible process with no solvent residue and low temperature operation for heat sensitive materials, commercial applications for fats and oils processing have been limited. Despite the common belief that commercial applications of supercritical fluid technology is restricted to high-value products due to the high investment cost, recent studies suggest that economics of the process become favorable at large capacities [6]. While some specialty oils are being extracted commercially, supercritical fluid extraction of commodity oils has not been commercialized yet. Supercritical fluid extraction suffers from a direct comparison with hexane extraction as this approach does not take into consideration the added advantage of supercritical fluid processing: simplification of the refining process [7]. Supercritical fluid extraction technology in fats and oils processing has to be considered not as an alternative to a single extraction step but as an alternative processing approach [8]. At a time when the conventional refining processes are being reexamined to maximize the retention of minor lipid components and the search to find an alternative to hexane is ongoing, $SCCO_2$ offers a versatile technology with the added advantage of "natural" processing. These factors coupled with technological developments in equipment design that would enable continuous processing of large batches of oilseeds may make $SCCO_2$ processing of fats and oils a commercial reality in the near future. Future research should focus on developing an alternative processing scheme for the processing of fats and oils, which would include various supercritical processing schemes and approaches as discussed throughout this thesis for the value-added processing of fats and oils.

7.4. REFERENCES

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